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## MEDIAL DEGENERATION OF THE AORTA AS SEEN IN TWELVE CASES OF DISSECTING ANEURYSM

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NEW YORK

Degenerative lesions in the media of the aorta have been described in cases of dissecting aneurysm for many years. Among the pioneers were Moriani,<sup>1</sup> Shennan and Pirie<sup>2</sup> and Furno.<sup>3</sup> Interest in the subject was given renewed vigor by Gsell,<sup>4</sup> who carried over to the aorta Wiesel's<sup>5</sup> observation of muscle necrosis in peripheral vessels. This was immediately followed by contributions from Erdheim,<sup>6</sup> who introduced a new concept—medial degeneration by mucoid degeneration rather than by antecedent muscle necrosis. Since then many others have added their efforts to the solution of the problem (Cellina,<sup>7</sup> Levinson,<sup>8</sup> Wolff,<sup>9</sup> Neuberger,<sup>10</sup> Moritz,<sup>11</sup> Klotz and Simpson,<sup>12</sup> Weise<sup>13</sup>).

The observations of Gsell were of particular importance. He described a form of medial degeneration which apparently began as focal necrosis of muscle. This was followed later by disintegration of elastic tissue and collagen, resulting in the formation of gaps. Healing, if it

From the laboratories of pathology of St Vincent's Hospital and Bellevue Hospital and the Office of the Chief Medical Examiner

1 Moriani, G. *Virchows Arch f path Anat* **202** 283, 1910

2 Shennan, T, and Pirie, J H H. *Brit M J* **2** 1287, 1912

3 Furno, A. *Arch di pat e clin med* **3** 26, 1924

4 Gsell, O. *Virchows Arch f path Anat* **1** 270, 1928

5 Wiesel, J. *Ztschr f Heilk* **8** 69, 1907

6 Erdheim, J. (a) *Virchows Arch f path Anat* **273** 454, 1929, (b) **276** 187, 1930

7 Cellina, M. *Arch ital di anat e istol pat* **2** 1105, 1931, *Virchows Arch f path Anat* **280** 65, 1931

8 Levinson, B. *Virchows Arch f path Anat* **282** 1, 1931

9 Wolff, K. *Virchows Arch f path Anat* **285** 1, 1932, **289** 1, 1933

10 Neuberger, K. *Ztschr f Kreislaufforsch* **24** 169, 1932

11 Moritz, A R. *Am J Path* **8** 717, 1932

12 Klotz, O, and Simpson, W. *Am J M Sc* **184** 455, 1932

13 Weise, W. *Beitr z path Anat u z allg Path* **93** 238, 1934

occurred, took place by the appearance of loose scar, very poor in collagen. Most striking was the absence of cellular reaction.

While in his first report Erdheim<sup>6a</sup> concurred with Gsell, in his second contribution<sup>6b</sup> he introduced a new form of medial degeneration, called by him "medionecrosis aortae idiopathica cystica." In this instance he believed that the disease began with the accumulation of abnormal quantities of mucoid material in the media, spreading as it increased beyond the confines of single interlamellar spaces until with the dissolution of previously normal muscle, collagen and elastic tissue, mucoid-filled spaces remained. Healing occurred by the formation of loose, nonvascularized scars or by regeneration of the original elements. At no time in the entire process was an inflammatory mechanism evident. Intima, adventitia and vasa vasorum played no visible role. In subsequent reports other students have described the medionecrosis of Gsell or the mucoid degeneration of Erdheim without clearly differentiating between the two.

It is the purpose of this paper to present a study of medial degeneration as encountered in 12 selected cases of dissecting aneurysm of the aorta. Eleven others were discarded either because tissue for study was no longer available or because the condition was complicated by another disease, such as syphilis or bacterial endocarditis. In 9 of the 12 cases the heart and aorta had been saved. In the remaining 3 cases the material available included the ruptured intima, making it possible to study the wall of the vessel in this region. In 10 cases the dissection and intimal rupture were recent, in the remaining 2, long standing. The intimal rupture was supravalvular in 10 cases and at the arch in 2.

#### METHOD OF STUDY

In 8 cases the entire aorta from the root to and including the arch was cut into serial blocks. Beyond, representative sections were taken every 3 to 4 cm. When these were cut transversely, they included the entire circumference of the vessel, when cut longitudinally, they extended from root to arch. Only in case 12 was it found necessary to make the sections small. However, enough was taken from representative locations to make the inclusion of the case in the series justifiable. Blocks were also cut from the myocardium and coronary arteries and from the valves when the latter were visibly diseased. The following stains were employed: hematoxylin and eosin, Mallory's phosphotungstic acid-hematoxylin, Weigert's stain for elastic tissue, Van Gieson's stain, Masson's trichrome stain. In some cases the Foot and Foot stain for reticulum was used. In a single instance the Von Kossa stain aided in demonstrating calcium. Thionine and cresyl echt violet were found useful for visualization of mucoid substances.

In order that the distribution of lesions might be studied better, a map of the aorta was made in each case and the different types of lesions were charted on it in code. Thus it was possible to note at a glance their number, distribution and relation to each other and to the point of intimal rupture (fig 1).

## RESULTS

In every case destructive noninflammatory lesions were found in the media of the aorta. The simplest and most common type, present in 10 of 12 cases, was characterized by focal loss of muscle (fig 2 *A*) and crowding together of elastic laminae. In only 3 of these 10 instances was it possible to recognize anuclear remains of muscle cells<sup>14</sup>. Lesions

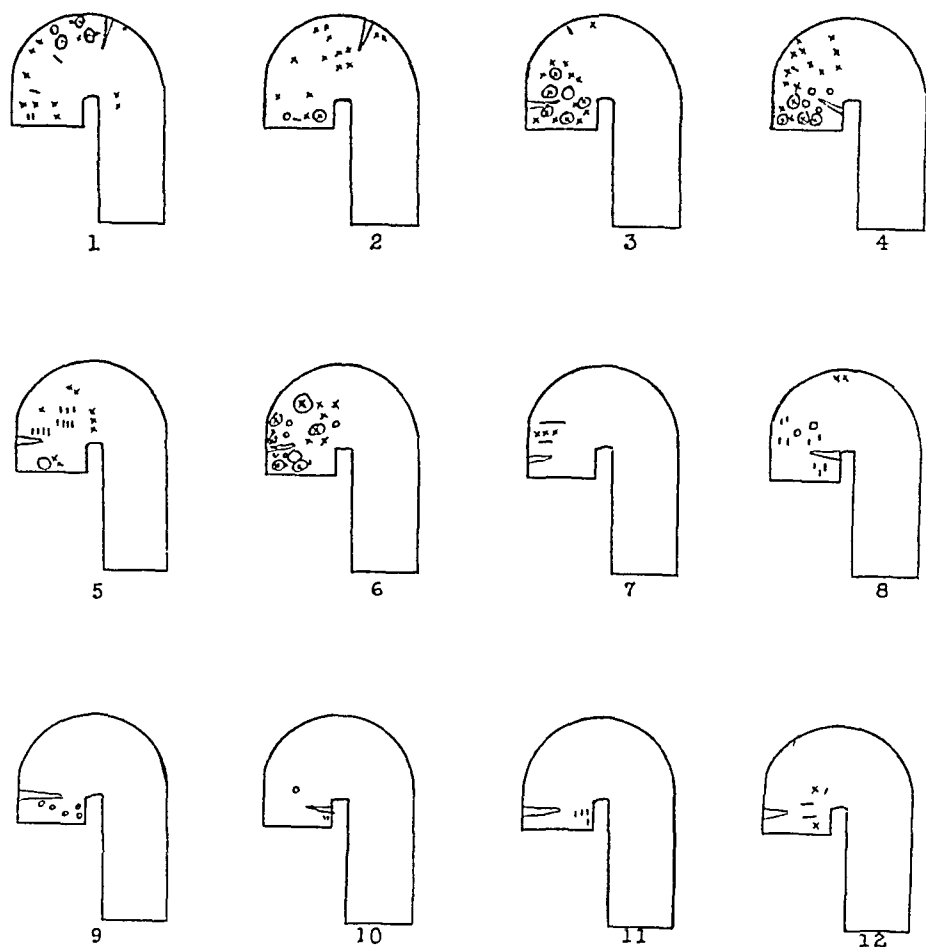


Fig 1—Diagrams of aortas from 12 patients with dissecting aneurysm, showing distribution of simple muscle loss ( $\backslash$ ), muscle and elastic tissue loss (circled  $\times$ ), cyst formation (o), scar formation (-) and medical regeneration (""') and the location of the intimal rupture (>)

of this type were found chiefly in the middle and inner thirds of the aorta from root to isthmus, adjacent to and beyond the region of intimal rupture, in dissected portions (cases 3, 4, 5, 6, 7, 8, 9 and 12) and non-dissected portions (cases 1 and 2) of the aorta. In case 1 only were they found in the descending portions as well. The severity as measured by

14 This finding is mentioned because it is used by Gsell as indicating necrosis

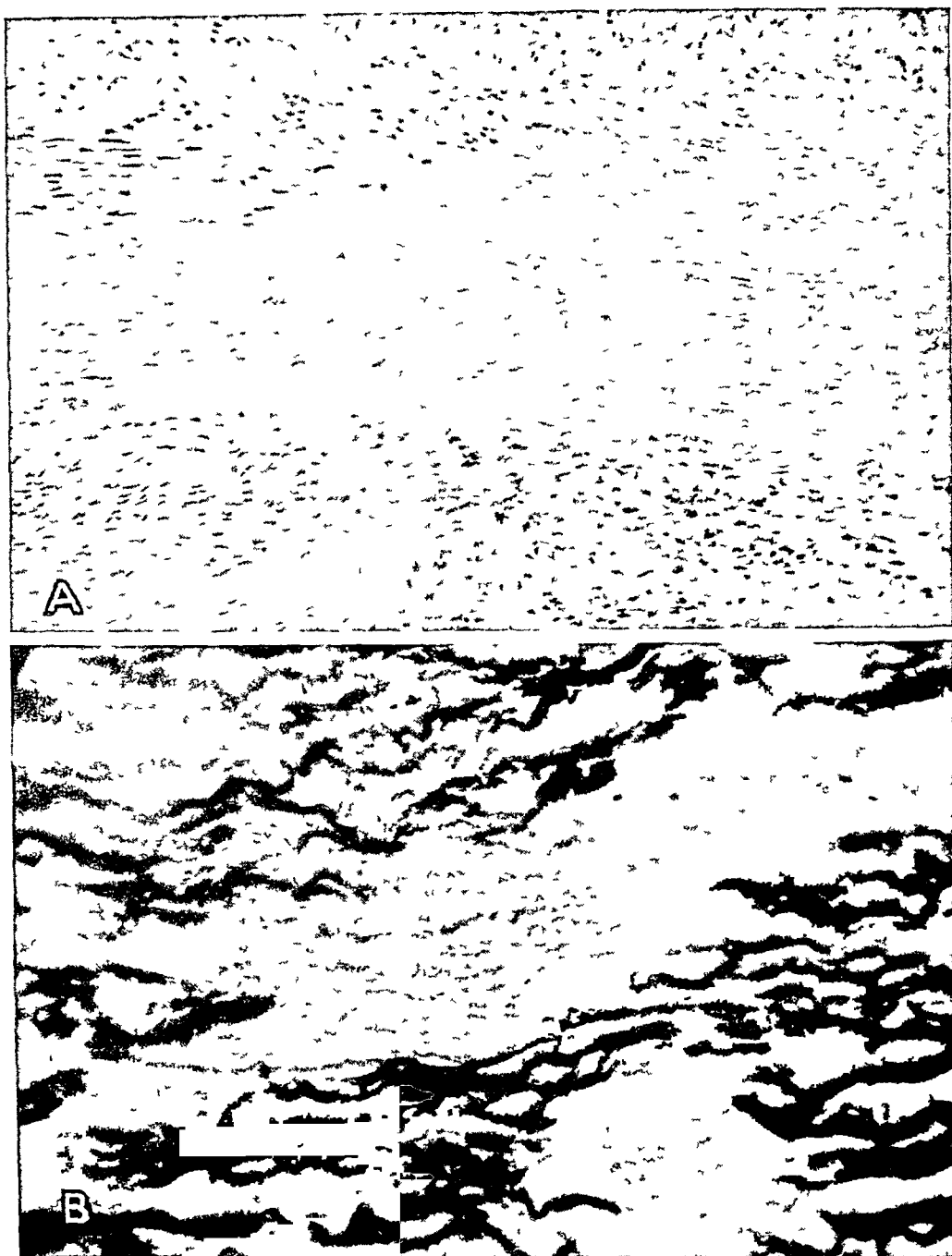


Fig 2—*A*, simple muscle loss, hematoxylin-eosin stain. Note the complete absence of cells in the midzone. *B*, more advanced degeneration, Weigert's stain for elastic tissue. In addition to the disappearance of muscle in this area, there are a thinning and loss of staining of the elastic lamellae.

size and number varied in different cases, being extreme in 4 cases (1, 2, 3 and 4), moderate in 3 (5, 6 and 7), mild in 3 (8, 9 and 12) and absent in 2 (10 and 11)

In 8 cases the lesions were of a more advanced type, characterized not only by loss of muscle but by degeneration of elastic and collagenous tissue as well. These tissues were found in all stages of disintegration, from thinning and loss of the tinctorial properties of elastic lamellae (fig 2 *B*) to the completion of the process with formation of mucoid-filled cavities bordered by normal media (fig 3 *A*)

The size of the lesions of this type varied. The damage was insignificant in some instances and in others it was visible on holding elastic tissue preparations up to the light. The largest lesion measured 3 to 4 mm long and occupied the entire width of the media. These lesions were moderately numerous in 6 cases (1, 3, 4, 6, 8 and 9), scant in 2 (2 and 10) and absent in 4 (5, 7, 11 and 12). Their distribution was more or less similar to that of simple muscle loss with the exception that in no case was a lesion of the advanced type present in the descending aorta. In 7 cases this type was found in the same vessel in which simple muscle loss was present. The predominance of each type in any one aorta varied. In 2 cases (1 and 2), the simple type predominated, in 2 others (3 and 4) both were equally conspicuous, and in 3 the more advanced lesion overshadowed the other (6, 8 and 9).

The third<sup>15</sup> type of lesion to be described was found in 8 cases (1, 5, 6 and 8 to 12). Its chief characteristic was the presence of variable numbers of muscle cells, scant or more numerous than normal, occupying areas devoid of elastic laminae (fig 3 *B* and 4 *A*). In some lesions the muscle cells apparently lay free in mucoid material, in others they were enmeshed in a network formed by loosely arranged collagenous tissue (fig 3 *B*). This type of lesion could be detected in hematoxylin-eosin preparations by the unusual direction of the long axes of the muscle cells, which ran perpendicularly or obliquely to muscle cells of adjacent normal media. In 2 cases (5 and 6) lesions of this type were moderately prominent, in 5, markedly so, and in 1, scant (1). In every instance they were observed in the lower half of the ascending aorta only. Such lesions constituted almost the sole abnormality in 3 cases (10, 11 and 12). In 3 others (5, 8 and 9) advanced degenerative changes were equally conspicuous. In the remaining 2 cases (1 and 6) the third type sank into insignificance alongside destructive lesions.

A fourth type of lesion, present in scant numbers in 4 cases, must be mentioned. It consisted of loosely constructed small fibrous scars

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<sup>15</sup> As I lean toward the theory of Erdheim, who felt that the lesion represented a regenerative phenomenon, I have captioned it in figure 1 as "medial regeneration."

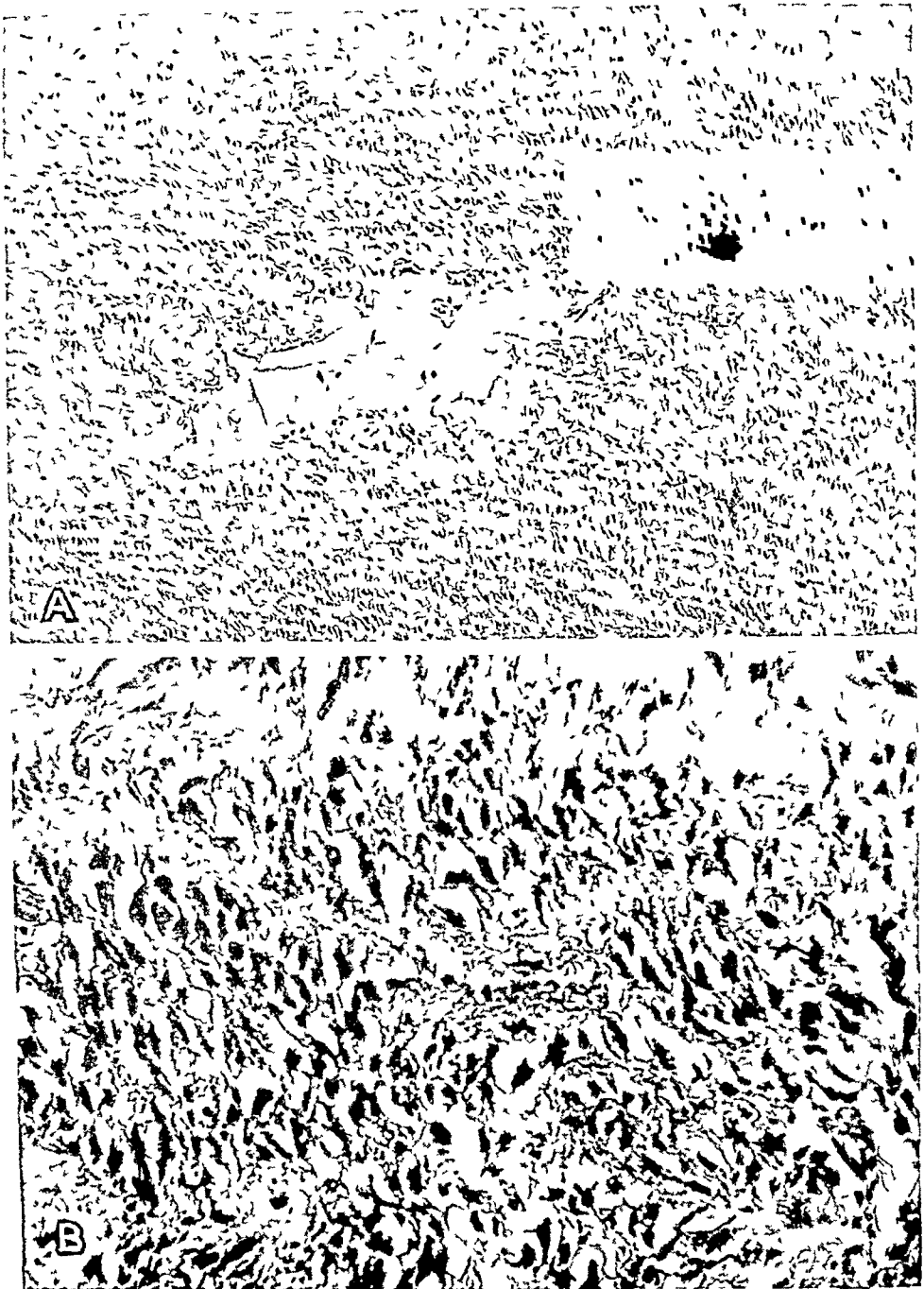


Fig 3—*A*, mucoid-filled cyst, surrounded by normal media, hematoxylin-eosin stain. This is an end stage of the process depicted in figure 2. *B*, area of regeneration, phosphotungstic acid-hematoxylin stain, high power magnification. Note that muscle cells are numerous and irregularly arranged. The supporting stroma consists of a loose collagen meshwork. Elastic tissue is not present. (See fig 4 *A*.)

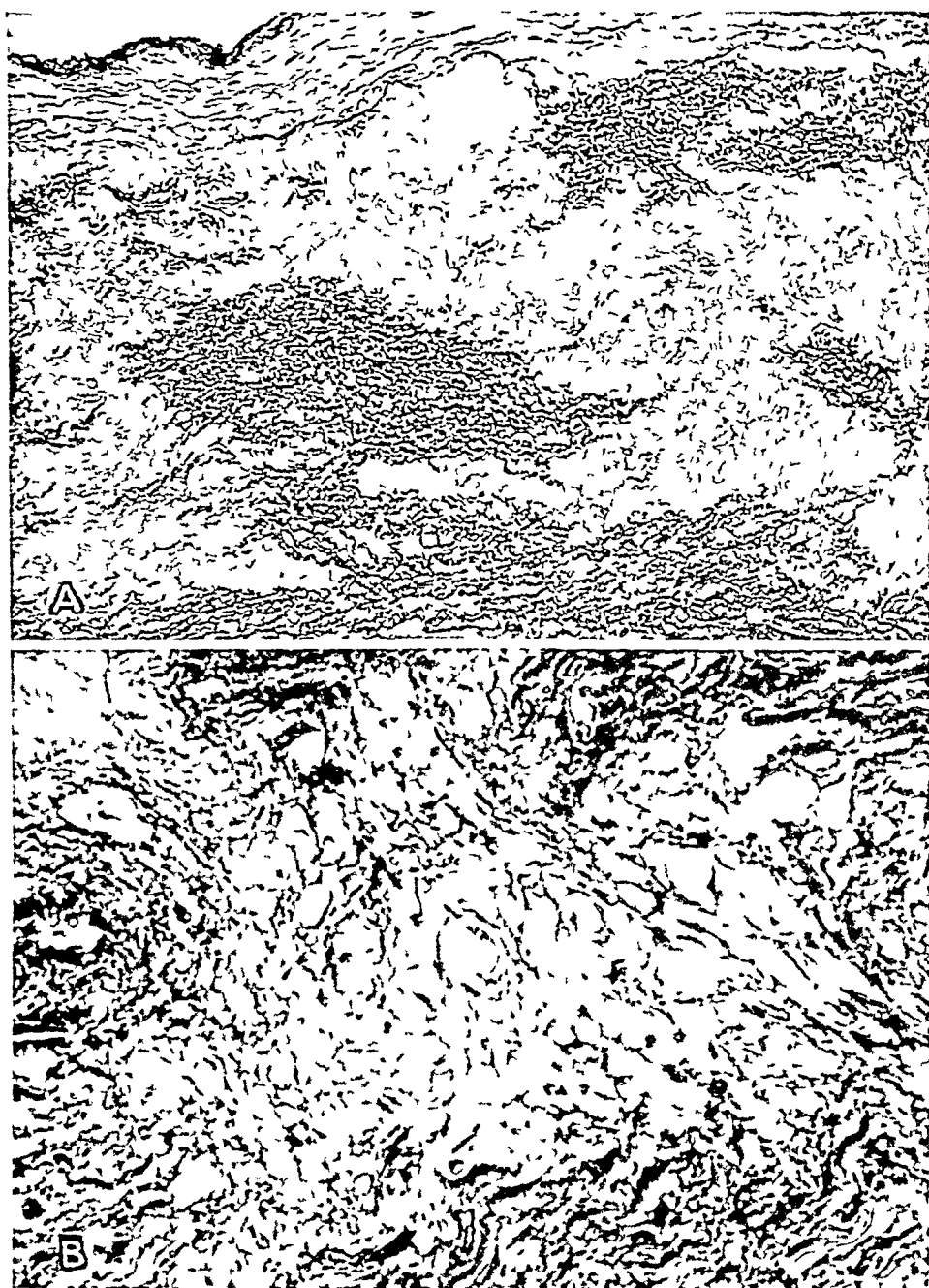


Fig 4—*A*, same lesion as in figure 3 *B*, illustrating the absence of elastic tissue, Weigert's stain for elastic tissue, low power magnification *B*, nonvascularized scar filling an area of degeneration, phosphotungstic acid-hematoxylin stain. Note looseness of the stroma. Fibroblasts are scant.



(fig 4 B) Though these scars were usually avascular, an occasional one contained wide endothelial-lined channels. Inflammatory reaction was completely absent from this type in every case.

No correlation could be established between intimal changes and the medial lesions. To be sure, some were subjacent to atherosclerotic plaques, both small and large, but these were the exceptions. It was impossible to discover adventitial thickening beneath the medial lesions as described by Gsell, except in the last case, and in that instance it was generalized rather than focal.

Changes in the vasa vasorum found in several cases were due to increase either of elastic or of collagenous tissue.

The cardiac findings in the series are as follows. The hearts of 9 patients were hypertrophied. Four of the patients from whom these hearts were removed had clinical records of hypertension. Fusion of the commissures of the aortic valve with thickening of the leaflets was noted in 5 hearts. One alone had demonstrable Aschoff bodies in the myocardium. One showed myocardial infarction.

The degree of coronary disease was insignificant except in 3 hearts. In case 7, in addition to atherosclerosis narrowing the lumen there was a marked inflammatory reaction of undetermined nature. In the third there was severe atherosclerosis but no occlusion.

#### COMMENT

Attempts to ascribe medial degeneration as seen in these cases to artefact or to change secondary to trauma or to nutritional insufficiency incidental to dissection can be dismissed. In the first place, it was found in nondissected portions of the aorta, secondly, it occurred rarely in the descending portion, which was as frequently dissected as the ascending aorta, and, finally, medial degeneration as herein described has been seen in our laboratory in routine autopsy material. One case was reported<sup>16</sup> in which the changes were more marked than in any instance in the present series. In that case there was aneurysm formation, but the wall was not dissected and not ruptured.

The disease is a distinct pathologic entity, different from any of the better known forms of aortic disease, such as syphilis and rheumatic fever, since the inflammatory changes characteristic in the latter two diseases are absent. In the same way, it differs from other less common forms of aortitis, such as those due to pyogenic infection, tuberculosis and periarteritis nodosa. This lack of inflammatory reaction in medial degeneration cannot be ascribed to inability of the organism to react, for in every instance in which hemorrhage had taken place in the adventitia numerous polymorphonuclear leukocytes were found

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16 Rottino, A. Arch Path 27:320, 1939

locally. Further, in older cases fibroblastic reaction was rich and new vascular channels abundant. The same proliferative phenomenon was noted on the dissected surfaces of the media, where in some instances a new intima was in the process of formation, seen par excellence in case 12, an instance of an old, healed lesion, where the reformed intima was dense, rich in collagen and densely packed with elastic fibrillae.

The finding of medial degeneration exclusively in the ascending aorta and arch in 11 of the 12 cases emphasizes Gsell's notation that it is essentially a disease of this portion of the vessel. Its progression from simple muscle loss to eventual cyst formation was a relatively simple matter to trace in 6 cases—an experience similar to that of Gsell's. Its inception in preliminary mucoid degeneration in the sense of Erdheim, on the other hand, could not be established. To be sure, in 4 cases there was degeneration of media with unusual collections of mucoid—surrounding abnormally arranged muscle or filling large spaces. To trace this to a lesion beginning with an overabundance of mucoid in single interlamellar spaces, however, was impossible.

A word must be said concerning the ability of smooth muscle to regenerate, since this potentiality is questioned by many pathologists. It is impossible in the cases herein presented to state that the evidence for this is the demonstration of visible mitosis or amitosis. However, when one sees in an area an increase in muscle cells, not ascribable to crowding, one is driven to the conclusion that they must have regenerated, possibly from nonaffected peripheral cells. Further proof is that in large areas of degeneration, as evidenced by loss of elastic tissue, one still finds muscle cells in profusion. Unless they are regenerated, one is compelled to accept the theory that they represent the original muscle which escaped the effect of a noxa capable of destroying elastic tissue.

The causes are entirely unknown. Certain factors are apparently associated sufficiently often to suggest themselves as predisposing. Age is one, for no patient in the present group was younger than 43, one was 51, and the rest were above 55. However, since in the literature there are reports of cases in which the ages were 21,<sup>10</sup> 23,<sup>5</sup> 25,<sup>4</sup> 28<sup>10</sup> and 29,<sup>17</sup> it appears that the necessity of old age is not absolute. A second finding of importance is the presence of pathologic changes in the heart in some form. This was observed in 11 of 12 specimens—9 with hypertrophy and 5 with lesions of the aortic valve. One third of the patients (4 of 12) had had hypertension. Four others had hypertrophied hearts with no valvular defects to account for the enlargement. Unfortunately, their blood pressure had not been taken.

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17 Narr, F. H., and Wells, A. H. *Am Heart J* 8:834, 1933.

The specific cause (or causes) remains problematic. Wiesel's<sup>5</sup> finding of medial degeneration in peripheral vessels of patients who died of acute infectious disease—sepsis or scarlet fever—has supplied one answer. Apparent support was given by Stoerk and Epstein,<sup>18</sup> who found the same change in the coronary vessels in fatal cases of grip. Gsell<sup>4</sup> suggested the importance of endogenous toxins. Three of his patients had renal insufficiency. Pappenheimer and Von Glahn<sup>19</sup> observed simple muscle loss in the aorta in several cases of rheumatic fever. They ascribed it to a nutritional disorder resulting from changes in and about the nutrient vessels. On the experimental side, degenerative lesions in the aorta have been reported in poisoning by nicotine, lead (Cellina<sup>7</sup>) and epinephrine (Lange<sup>20</sup>), in electrical and thermal stimulation of the wall (Erdheim<sup>6b</sup>), also, in consequence of feeding pulverized organs to rabbits (Steinbiss<sup>21</sup>). Erdheim, noting how unrelated the assumed causes were, came to the conclusion that all of them in some way were responsible for overproduction of epinephrine. In the proper patient this caused prolonged contraction of vasa vasorum and ischemic necrosis of the media. My material offers no help to any of the theories.

#### SUMMARY

Medial degeneration was studied in the aorta in 12 selected cases of dissecting aneurysm. The lesion was characterized by loss of muscle, elastic tissue and collagen, lack of inflammatory reaction and healing by loose scar formation and by regeneration of muscle and elastic tissue. Loss of muscle appears to be the initial lesion. The degeneration of the remaining medial elements follows.

Medial degeneration as described by Erdheim could not be established to be present in our material.

Medial degeneration is essentially a disease of the ascending aorta and arch.

The specific cause remains unknown. Possible predisposing causes are old age, heart disease, hypertension.

18 Stoerk, O., and Epstein, E. *Frankfurt Ztschr. f. Path.* **23** 163, 1920.

19 Pappenheimer, A. M., and Von Glahn, W. C. *J. M. Research* **44** 489, 1924.

20 Lange, F. *Virchows Arch. f. path. Anat.* **248** 463, 1924.

21 Steinbiss, W. *Virchows Arch. f. path. Anat.* **212** 152, 1913.

# TRICHINELLA SPIRALIS

## 1 INCIDENCE OF INFECTION IN MAN, DOGS AND CATS IN THE NEW ORLEANS AREA AS DETERMINED IN POSTMORTEM EXAMINATIONS

WILLI SAWITZ, M D

NEW ORLEANS

The incidence of infection with *Trichinella spiralis* is higher in the United States than in any other country<sup>1</sup> Practical methods for the reduction and control of the infection depend on knowledge of the incidence in the various hosts and the epidemiologic role these hosts play, in order that the most important transmission lines of this parasite may be interrupted

Any mammal which eats trichinous muscle is apt to contract trichinella infection and on being eaten itself may act as a transmitter *Trichinellas* occur not only in man but in the domestic and the wild hog, the captive and the wild bear, the mouse, the rat the dog, the cat, the mongoose, the fox, the badger, the marten, the marmot, the polecat (skunk, fitchet), the raccoon, the ichneumon (*Helix ichneumon*) and the hippopotamus The transmission of *T spiralis* from host to host is diagrammatically shown in figure 1 Only those animals are included which play a part in the epidemiology of trichinosis in the New Orleans area

Man acquires trichinella infection by eating infected pork (exceptionally bear meat) Hogs acquire it by eating infected pork scraps or infected rats Rats acquire it by eating other infected rats, infected pork scraps or infected carcasses of dogs or cats Dogs become infected by eating infected pork scraps or infected rats Cats obtain the infection by eating infected pork scraps, rats or mice, which in turn acquire it by eating infected pork scraps

In order to ascertain the prevalence of *T spiralis* in the New Orleans area, a survey of its incidence in man, hogs, rats, mice, dogs and cats has been conducted This paper deals with the incidence in man, dogs and cats The incidence in hogs, rats and mice has been studied in this laboratory by C E Peres, and his findings will be published later

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From the Parasitology Laboratory, Department of Tropical Medicine, Tulane University of Louisiana

This work was supported by a grant from the Committee on Scientific Research of the American Medical Association

1 Sawitz, W Pub Health Rep 53 365, 1938

In 1936 Hinman,<sup>2</sup> using the artificial digestion method, examined 2 square inches (12 sq cm) of each of 200 human diaphragms obtained at autopsies at the Charity Hospital in New Orleans and found 7 diaphragms infected with *T spiralis*—an incidence of 3.5 per cent. This figure has been regarded as too low by McNaught and Anderson<sup>3</sup> and by Hall and Collins<sup>4</sup> on the ground that larger amounts of muscle, additional muscles or additional methods of examination might have yielded a higher incidence of infection.

#### METHODS

Human diaphragms and pectoral muscles were obtained at unselected routine necropsies in the Charity Hospital and the Touro Infirmary. Diaphragms of dogs

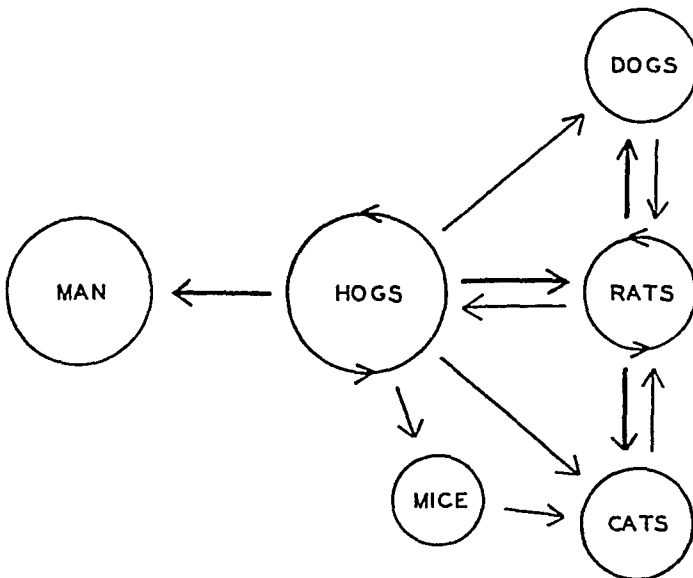


Fig. 1—Transfer of trichinellas from host to host in the New Orleans area

and cats were obtained from animals studied in various departments of Tulane University School of Medicine.

The muscles were separated from fat, and about half a gram of each muscle was pressed between two slides in a compressor similar to that used by the United States Bureau of Animal Industry. The piece of muscle was examined for larvae of *T spiralis* under a dissecting binocular microscope, with a  $\times 4$  ocular and a  $\times 18$  objective.

In addition to the compressor method, the digestion method of collecting larvae of *T spiralis* was employed—a method devised by Thornbury<sup>5</sup> in 1897 and first used to ascertain the incidence of trichinella infection in man by Queen,<sup>6</sup> in 1931.

2 Hinman, E. H. *New Orleans M & S J* **88** 445, 1936.

3 McNaught, J. B., and Anderson, E. V. *J A M A* **107** 1446, 1936.

4 Hall, M. C., and Collins, B. J. *Pub Health Rep* **52** 468, 1937.

5 Thornbury, F. J. *Univ M Mag* **10** 64, 1897.

6 Queen, F. B. *J Parasitol* **18** 128, 1931.

The muscles were ground and weighed, and 100 cc of artificial gastric juice was added to each 10 Gm of ground tissue. The maximum amount of diaphragm used was 170 Gm, the smallest, 10 Gm, the average, 50.8 Gm. The maximum amount of pectoral muscle used was 150 Gm, the smallest, 5 Gm, the average, 34.6 Gm. The artificial gastric juice was a solution of 1 per cent pepsin and 0.5 per cent hydrochloric acid. Digestions were carried on for twelve to sixteen hours in an incubator at 37.5 C (overnight). The fluid was then strained through two layers of cheesecloth into a funnel 8 inches (20 cm) in diameter, with a rubber tube and screw clamp attached to the funnel stem. After three hours 25 cc

TABLE 1—*Findings in Infected Human Diaphragms and Pectoral Muscles by Means of the Compressor and Digestion Methods*

Case	Diaphragms					Pectoral Muscles				
	Com pressor Method	Digestion Method				Com pressor Method	Digestion Method			
		Amount of Tissue Exam ined, Gm	Larvae	Calcified Cysts	Number per Gram		Amount of Tissue Exam ined, Gm	Larvae	Calcified Cysts	Number per Gram
1	0	50	0	0	0	+	10	1	0	0.1
2	0	30	2	0	0.07	0	15	1	0	0.07
3	0	20	0	1	0.05	0	10	0	0	0
4	0	50	0	3	0.06	0	20	0	0	0
5	0	80	2	0	0.01	0	10	0	0	0
6	0	50	0	21	0.4	0	10	0	6	0.6
7	0	50	0	31	0.6	0	15	0	6	0.4
8	0	50	6	0	0.12	0	15	2	0	0.13
9	0	40	0	38	0.95	0	15	0	7	0.47
10	0	60	0	3	0.05	0	10	0	1	0.1
11	0	50	2	0	0.04	0	70	0	0	0
12	0	110	8	1	0.07	0	55	3	1	0.07
13	0	30	17	1	0.6	0	35	7	3	0.29
14	0	40	0	6	0.15	0	10	0	0	0
15	0	90	0	9	0.1	0	10	0	0	0
16	0	27	0	0	0	0	60	2	0	0.03
17	0	60	0	2	0.03	0	50	0	0	0
18	0	60	2	2	0.07	0	60	0	0	0
19	0	35	0	10	0.28	0	30	0	1	0.03
20	0	60	0	25	0.42	0	60	0	0	0
21	0	25	0	0	0	0	35	1	0	0.29
22	0	25	0	9	0.36	0	45	0	3	0.06
23	0	85	0	13	0.15	0	30	0	0	0
24	+	90	497	0	5.52	Not examined				

of the fluid was drawn off into a Petri dish which had been ruled in squares. Each square had been numbered by means of a diamond pencil in order to facilitate counting. The fluid in the Petri dish was examined with a dissecting binocular microscope, using a  $\times 4$  ocular and a  $\times 18$  objective. Trichinellas found were differentiated as living larvae or calcified cysts, and the number of each was counted. The examination was repeated with more fluid until two successive samples were negative.

#### INCIDENCE IN MAN

Examinations were made of tissues obtained at 400 unselected routine autopsies, including 200 previously reported.<sup>7</sup> Larvae of *T. spiralis* were found in 24 cases—an incidence of 6 per cent.

The quantitative data on the findings in the diaphragms and pectoral muscles by the compressor and digestion methods are presented in table 1

Of the 24 instances of trichinella infection, 2 were found by the compressor method (cases 1 and 24), while all were found by the digestion method. In view of the fact that only half a gram of muscle was examined by the compressor method, it was to be expected that the cases detected by this method would be those in which at least 1 larva was present in half a gram of tissue. Cases with less than 1 larva in half a gram of tissue would be expected to be discovered according to the chances of probability. Only 1 of the 24 cases (case 24) showed more than 1 larva in half a gram of tissue by the digestion method, and this case was found by the compressor method. The case in which the digestion method revealed 0.1 larva in 1 Gm (case 1) was probably found by chance. In the digestion method a much larger amount—on the average 50.8 Gm of diaphragm and 34.6 Gm of pectoral muscle—was used, and the larvae present were collected in the sediment. This technic serves as a concentration method.

Of the 23 cases in which both the diaphragm and the pectoral muscle were available, the parasites were shown in the diaphragm in 20—an incidence of 87 per cent. In 13 cases the parasites were shown in the pectoral muscle—an incidence of 56.5 per cent. In other words, if the diaphragm alone had been examined, 13 per cent of the cases of infection would have been missed, and 43.5 per cent if only the pectoral muscle had been examined. According to Thoinbury,<sup>5</sup> the diaphragm is considered the best material in which to discover the larvae of *T. spiralis*. He found them in the diaphragms of 76.6 per cent of 1,043 trichinous swine, while 23.4 per cent of the infected swine had the larvae only in loin or neck muscles or both. No such studies seem to have been made in man. The comparative figures in the present study indicate that the diaphragm is a better site for discovering the larvae than is the pectoral muscle and that the distribution in man may be similar to that in swine. The figures 76.6 per cent for hog diaphragms and 87 per cent for human diaphragms might have been still closer if additional muscles had been examined, since then the figure for the total incidence might have been greater, and thus the percental incidence in the diaphragms lower.

To facilitate comparison of the quantitative findings in diaphragms and pectoral muscles, the cases are arranged in table 2 according to the intensity of infection.

The average number of trichinellas found per gram of diaphragm in the 10 cases in which both the diaphragm and the pectoral muscle were infected was 0.35, whereas the average number in the pectoral muscle was 0.22 per gram. This indicates that the diaphragm is not

only qualitatively but also quantitatively the better material in which to search for these parasites. The value of the surgical removal of a piece of pectoral muscle for diagnostic purposes is not diminished by these results, since the biopsy method is employed in cases of clinical trichinosis, in which a heavy general infection and thus infiltration of the pectoral muscles may be expected.

Hall and Collins<sup>4</sup> advanced the theory that the rapidity with which the larvae die and calcify is proportional to the intensity of the infection. They found only living larvae in the majority of infections with less

TABLE 2—Order of Cases with Regard to Increasing Intensity of Infection in the Diaphragmatic Tissue

Case	Age of Patient	Larvae Found per Gram in		Living (L) or Calcified (C)
		Diaphragm	Pectoral Muscle	
1	43	0 00	0 1	L
16	44	0 00	0 03	L
21	45	0 00	0 29	I
5	40	0 01	0	L
17	50 60	0 03	0	C
11	60	0 04	0	L
3	61	0 05	0	C
10	48	0 05	0 1	C
4	65	0 06	0	C
2	29	0 07	0 07	L
12	32	0 07	0 07	L, C
18	49	0 07	0	L, C
15	51	0 1	0	C
8	60	0 12	0 13	L
14	57	0 15	0	C
23	76	0 15	0	C
19	59	0 28	0 03	C
22	73	0 36	0 06	C
6	70	0 4	0 6	C
20	73	0 42	0	C
7	56	0 6	0 4	C
13	60	0 6	0 29	L, C
9	36	0 95	0 47	C
24	48	5 52	Not examined	L

than 1 larva per gram, in infections with 1 to 10 larvae per gram, some of the larvae were living and some were dead, in cases with more than 10 larvae per gram, mixed infections or only calcified cysts were found. In 23 of the cases there was less than 1 larva per gram. In 7 of these only living larvae were found, in 3 a mixed infection and in 13 only calcified cysts. In the single case in which more than 1 larva per gram occurred, only living ones were observed. However, on dividing the cases into two groups, one the cases with less than 0 1 trichinella per gram and the other those with more, one notes in the first group 12 cases, 6 (50 per cent) of which showed only living larvae, 2 (16 6 per cent) a mixed infection and 4 (33 3 per cent) calcified cysts. Of the 12 cases of the second group, 2 (16 6 per cent) showed only living larvae, 1 (8 3 per cent) a mixed infection and 9



(75 per cent) calcified cysts. These figures seem to support the theory of Hall and Collins.

The age, sex, color and nativity of the patients are recorded in table 3.

The incidence of infection in the first 100 cases examined was 4 per cent, in the second 6 per cent, in the third 7 per cent and in the fourth 7 per cent. A variation of 4 to 7 per cent in 100 cases is in the same range as that found by Nolan and Bozicevich<sup>8</sup> in their study of 1,000 diaphragms, their lowest incidence being 12 per cent in one series of 100 cases, their highest 24 per cent. In addition, the

TABLE 3—*Age, Sex, Color, Nativity and Occupation of the Persons Found Infected*

Person	Age	Sex	Color*	State of Birth	Occupation
1	43	M	W	Texas	Bartender
2	29	F	N	La	Housewife
3	61	M	W	La	Horse trainer
4	65	F	W	La	Housewife
5	40	M	W	La	None
6	70	M	N	?	None
7	56	F	W	La	Housewife
8	60	M	W	La	Laborer
9	36	M	N	La	Laborer
10	48	M	N	La	Laborer
11	60	F	N	La	Housewife
12	32	M	N	La	Laborer
13	60	M	N	N C	None
14	57	F	W	La	Seamstress
15	51	F	N	La	Housewife
16	44	F	N	La	Cook
17	50 60	M	N	La	Laborer
18	49	F	N	La	None
19	59	M	W	La	?
20	73	M	N	Texas	None
21	45	F	N	La	Truck driver
22	73	F	N	La	Farmer
23	76	M	W	La	None
24	48	M	N	La	Laborer

\* W indicates white, N, Negro

incidence will vary, according to the chances of probability, with age, sex, race, nationality and economic status, as demonstrated by Hall and Collins.<sup>9</sup>

The mean average age of the first 100 patients whose tissues were examined post mortem was 44.52 years, of the second 100 patients 44.9 years, of the third 100 patients 46.35 and of the fourth 100 patients 44.76. In addition, 13 patients included in the first group were of a higher economic status than the others. These factors may account for the lower incidence in the first 100 patients.

In the 24 patients found infected, no clinical symptoms of light or chronic trichinella infection had been noted. This is in accordance

<sup>8</sup> Nolan, M. O., and Bozicevich, J. Pub. Health Rep. **53** 652, 1938.

<sup>9</sup> Hall, M. C., and Collins, B. J. Pub. Health Rep. **52** 512, 1937, footnote 4.

with the findings in previous surveys in other areas. The highest eosinophil count in the 24 cases of infection was 1 per cent. However, in only 4 of the 24 cases were blood counts available. The findings in these 4 cases are given in table 4.

The small number of cases in which blood counts were available does not warrant the conclusion that small numbers of trichinellas, even living ones, do not stimulate or sustain eosinophilia. The time that elapses between the exposure to infection and the examination of the blood plays an important part, since the number of eosinophils

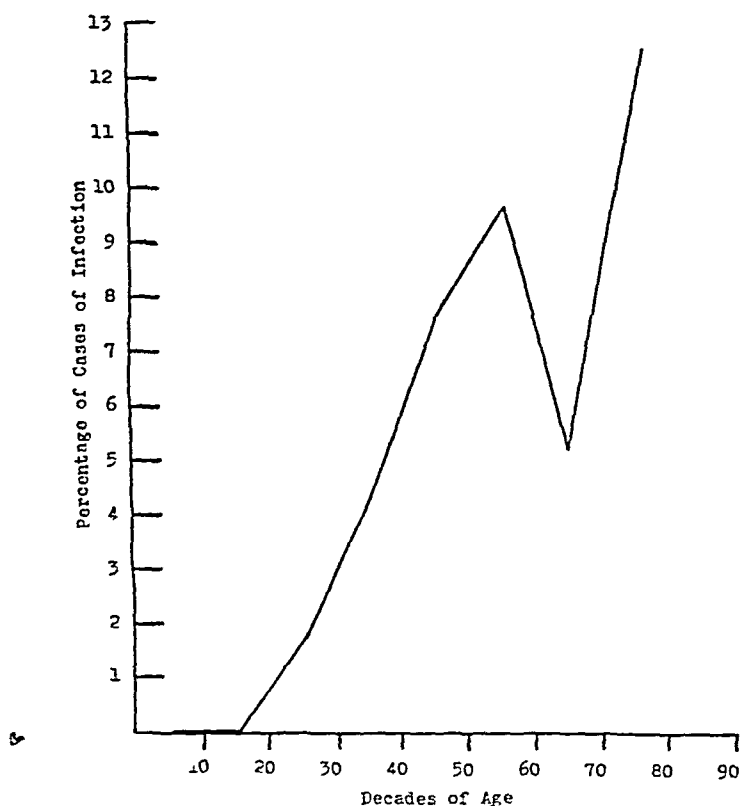


Fig 2—Percent incidence of trichinella infection by decades of age

decreases in the course of time after it has reached a maximum in the acute stage. In a case of clinical trichinosis which was followed up, the percentage of eosinophils was 36 per cent four weeks after exposure, 10 per cent after one year and 2 per cent after two years. This suggests that in these 4 cases infection occurred at least two years before death.

The distribution of the cases by decades of age is shown in table 5.

Figure 2 shows the curve of the percental distribution of the cases of infection by decades of age. It shows an increasing incidence of trichinella infection with increasing age. Since the chances of becoming

infected increase with age, the rising curve is understandable In the group who were examined in the first decade are included 2 stillborn infants and 3 babies under 1 year of age, all 5 found uninfected The negative findings in the first and second decades are in agreement with the report of McNaught and Anderson,<sup>3</sup> who did not find infection in

TABLE 4—*Eosinophil Counts in Four Cases*

Case	Total White Cell Count	Eosinophils, Per Cent	Larvae of Trichinella Found per Gram
2	10,500	0	0 07, living
20	7,000	0	0 42, calcified
21	11,400	0	0 29, living
22	10,400	1	0 36, calcified

TABLE 5—*Distribution of the Cases by Decades of Age*

Decades of Age	Cases in Which Tissues Were Examined	Cases in Which Tissues Were Found Infected	
		Number	Percentage
0 10	17	0	0
11 20	18	0	0
21 30	54	1	1 85
31 40	69	3	4 34
41 50	76	6	7 89
51 60	82	8	9 76
61 70	58	3	5 17
71 80	24	3	12 5
81 90	1	0	0
Unknown	1	0	0
	400	24	6

TABLE 6—*Distribution of Trichinella Infection According to Sex and Color*

Race	Males Examined	Males Infected		Females Examined	Females Infected		Total		
		Number	Percentage		Number	Percentage	Number Examined	Number Infected	Percentage Infected
White	105	6	5 71	59	3	5 08	164	9	5 43
Negro	131	8	6 10	105	7	6 66	236	15	6 36
	236	14	5 93	164	10	6 09	400	24	6

a single person under 25 years of age No explanation can be offered for the drop in the seventh decade A similar drop was found by Hall and Collins <sup>4</sup> in the eighth decade

The distribution of trichinella infection according to sex and color is shown in table 6

The incidence in males (5 93 per cent) and that in females (6 09 per cent) are close This is in agreement with the reports of McNaught and Anderson <sup>3</sup> and Hall and Collins,<sup>9</sup> who did not find any essential

difference in the incidence in these two groups. Analyzing these figures and considering the incidence for white males, white females, Negro males and Negro females separately, one finds the highest incidence in Negro females and the lowest in white females. Hall and Collins<sup>9</sup> also found the highest incidence in Negro females, the lowest incidence, however, they found in Negro males. They explained the sex difference (19.6 per cent in Negro females and 4.2 per cent in Negro males) by the fact that Negro females working as cooks are more exposed to infection through their tasting of raw and undercooked pork and pork products while engaged in their tasks. In the present series the number of those listed as cooks or servants is only 3. While Negro people may be less "trichinella conscious," they usually prepare their meat in a rather overdone manner, whereas white women, although they know more about the danger, like their pork rather underdone. Since in this series no sea-going persons are listed, a group in which Hall and Collins showed the highest incidence among white persons, it is understandable that the high incidence found by these observers in white males is not matched in the present series. The differences in the groups based on sex and color are not enough to be significant.

Almost all of the 400 persons whose diaphragms and pectoral muscles were examined belonged to the group of low economic status. Hall and Collins<sup>9</sup> found an incidence of 9.6 per cent in persons of high economic status and 14.6 per cent in persons of low economic status. They explain the difference by the food habits in these groups. If the ratio of incidence in their two groups is applicable to the incidence in the population of the New Orleans area, the 6 per cent incidence found in persons of low economic status corresponds to a 3.9 per cent incidence in the group of high economic status. The 13 persons of high economic status examined in the present series were not found infected.

Infection was found in 20 of 339 persons listed as natives of Louisiana, in 2 of 3 from Texas and in 1 of 4 whose nativity could not be ascertained. None of the 3 persons from Germany, 3 from Italy, 2 from France, 1 from Ireland and 1 from Greece was found infected. Since it is not known how long they lived in their native country and in this area, no significance can be attributed to this relationship of trichinella infection and nativity.

#### INCIDENCE IN DOGS

The diaphragms of 300 dogs were examined by the digestion method. Larvae of *T. spiralis* were recovered in 4—an incidence of 1.3 per cent. All infections were light, only living larvae were found—no calcified cysts.

Brumpt<sup>10</sup> mentioned that in 1913 Hjoitlund in Denmark found trichinella infection in 0.4 per cent of dogs. According to von Ostertag,<sup>11</sup> the incidence among dogs in Germany examined in 1904 to 1934 was 0.2 per cent. Yugawa<sup>12</sup> in Manchuria found 14 trichinous dogs among 179 street dogs of Liaoyang and Mukden in 1934—an incidence of 7.97 per cent. No survey of dogs for trichinellas seems to have been made previously in the United States.

#### INCIDENCE IN CATS

The diaphragms of 90 cats were examined by the digestion method. Nine cats were found infected—an incidence of 10 per cent. Five of these cats were heavily infected, as shown by the number of recovered trichinellas. Only living larvae were found—no calcified cysts.

In Denmark Hjoitlund<sup>13</sup> found an incidence of 2 per cent in cats, in Rumania Cernianu<sup>13</sup> found an incidence of 8 per cent. In 1937 Ch'in<sup>14</sup> found trichinellas in the tongue of a cat in Mukden. In the United States Riley<sup>15</sup> examined 25 cats in Saint Paul, Minn., and found 3 infected—an incidence of 12 per cent. On another occasion he found 1 cat infected among 5 examined, i. e., an incidence of 20 per cent. Riley concluded that cats play a considerable part in the perpetuation of endemics of *T. spiralis*. A survey of cats in order to ascertain the incidence of *T. spiralis* in them and the possible importance of cats in the epidemiology of *T. spiralis* does not seem to have been made. In passing, it is of interest to note that the first infection of lower animals with *T. spiralis* was found in cats by Herbst,<sup>16</sup> in 1845.

#### COMMENT

Since the majority of the persons from whom the examined tissues came belonged to the group of low economic status, this series does not represent a cross section of the population. Following Hall's<sup>9</sup> ratio between the groups of low and high economic status, the 6 per cent incidence in this group of low economic status would correspond to a 3.9 per cent incidence in the group of high economic status, the incidence in the entire population could be computed as being between 4 and 6 per cent. Although Hinman<sup>2</sup> examined approximately the same group of the population, his positive findings indicated an incidence

10 Brumpt, E. *Precis de parasitologie*, Paris, Masson & Cie, 1936, p. 1047.

11 von Ostertag, R. *Leitfaden für Trichinenschauer*, ed. 6, Berlin, Verlagsgesellschaft von Richard Schoetz, 1935, p. 61.

12 Yugawa, T. *J. Orient. Med.* **21**: 88, 1934.

13 Cited by Brumpt<sup>10</sup>.

14 Ch'in, Y. T. *Chinese M. J.* **51**: 500, 1937.

15 Riley, H. A. *Ann. de parasitol.* **6**: 477, 1928.

16 Herbst, cited by Staubli, C. *Trichinosis*, Wiesbaden, J. F. Bergmann, 1909.

of 3.5 per cent as compared with the 6 per cent which is reported here. If diaphragms alone had been examined, as in Hinman's series the incidence reported here would have been 5.25 per cent. The difference between 3.5 and 5.25 is probably due to the larger amounts of muscle examined in the present study since Hinman used only approximately 10 Gm of diaphragm. He should therefore have found only those cases in which there was at least 1 larva in 10 Gm or 0.1 larva per gram of tissue. Applying this method to the present series, I find the incidence to be only 4.25 per cent, a figure not far from the 3.5 per cent reported by Hinman. Thus the data of the present investigation support Hinman's conclusion that the incidence of trichinella infection in the New Orleans area is the lowest in the United States so far as surveys made up to the present show.<sup>1</sup>

The examination of dogs and especially that of cats showed that *T. spiralis* is present in this area. While man may acquire the infection away from his home area, cats, especially, do not migrate far. The incidence in cats therefore is considered a true index of endemicity of trichinella infection and an indication of the possibility of human infection.

The conclusions to be drawn from these findings will be discussed in a subsequent paper on the epidemiologic aspects of trichinella infection after the survey of the various hosts in the New Orleans area is completed.

#### SUMMARY

Examination of human diaphragms and pectoral muscles obtained in four hundred routine unselected necropsies disclosed 24 cases of infection with *T. spiralis* in the New Orleans area—an incidence of 6 per cent. The compressor method detected 2 cases; the digestion method, 24. Of the 23 cases in which both the diaphragm and the pectoral muscle were available, the diaphragm was found infected in 20 cases or 87 per cent and the pectoral muscle in 13 cases or 56.5 per cent. Surveys in which diaphragms only are examined would thus miss 13 per cent of the cases. The average number of larvae of *T. spiralis* found in the diaphragm was 0.35 per gram; the average number in the pectoral muscle, 0.22 per gram. The diaphragm is therefore, not only qualitatively but also quantitatively the better tissue for examination. No history of clinical symptoms of trichinosis was found in any of the 24 cases. With increasing age the incidence of trichinella infection increased. The highest incidence was found in Negro females (6.66 per cent), the lowest, in white females (5.8 per cent).

The incidence of trichinella infections in 300 dogs in the New Orleans area was found to be 1.3 per cent; the incidence in 90 cats was found to be 10 per cent. The incidence in cats is considered to serve as an indicator of the endemicity of trichinella infection in an area.

# SCLEROSIS OF THE SUPERIOR VENA CAVA IN CHRONIC CONGESTIVE HEART FAILURE

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AND

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It has been generally accepted <sup>1</sup> that persistent local increase of intra-arterial pressure is a prime cause of arteriosclerosis in both the greater and the lesser circulation. Sclerosis of the veins in the course of increased intravenous pressure of long duration has been less frequently described, largely because chronically increased intravenous pressure is relatively uncommon.

While direct measurements of the pressure in the human superior and inferior venae cavae are not available, it is well established that in patients suffering from chronic congestive heart failure the pressure in the peripheral veins may be greatly increased. Since the pressure in these veins is transmitted directly from the heart, the pressure in the venae cavae must also be elevated. One of us (H G) <sup>2</sup> demonstrated sclerosis of the inferior vena cava and of the hepatic veins in chronic congestive heart failure. The purpose of this study was to determine whether similar changes occur in the superior vena cava.

## REVIEW OF THE LITERATURE

Moschcowitz <sup>1</sup> is of the belief that increased tension is the sole cause of both arteriosclerosis and phlebosclerosis. Ljungdahl <sup>3</sup> as well as Miller <sup>4</sup> reported the frequent occurrence of pulmonary arteriosclerosis associated with hypertension of the lesser circulation occurring in mitral stenosis. The former noted that in many of the cases the pulmonary veins also showed sclerosis. He observed greater sclerosis in the arteries than in the veins and attributed the greater changes to the higher pres-

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From the Medical and Laboratory Divisions of Montefiore Hospital

1 Moschcowitz, E. *Am J M Sc* **178** 244, 1929, Phlebosclerosis of the Hepatic Veins, in *Contributions to the Medical Sciences in Honor of Dr Emanuel Libman by His Pupils, Friends and Colleagues*, New York, International Press, 1932, vol 2, p 857

2 Gross, H. *Arch Path* **23** 457, 1937

3 Ljungdahl, M. *Untersuchungen uber die Arteriosklerose des kleinen Kreislaufs*, Wiesbaden, J F Bergmann, 1915

4 Miller, H R. *M Clin North America* **9** 673 1925

sure within the arteries as well as to nutritional disturbances. He called attention to the similarity of phlebosclerosis to arteriosclerosis and noted the relationship between the degree of increased tension within the lumen and the degree of dilatation of a vessel. While Kaya<sup>5</sup> noted the frequent association of phlebosclerosis and chronic stasis, he doubted that congestive heart failure alone could cause phlebosclerosis in young persons. Schilling<sup>6</sup> also observed phlebosclerosis at sites of increased pressure and reported that in a series of 75 cases phlebosclerosis was due to congestion in 33. Waaler<sup>7</sup> observed hyaline plaques in the superior vena cava and in the right auricle. He regarded them as representing either the final and healed stage of lesions of acute rheumatic fever or sclerosis from long-standing heart disease. Allen and Page,<sup>8</sup> however, denied that sclerosis of the inferior vena cava occurred secondary to chronic congestive heart failure.

Sack<sup>9</sup> was of the belief that phlebosclerosis is part of a generalized vascular disease. Schilling<sup>6</sup> and Simmonds,<sup>10</sup> however, insisted on the independence of phlebosclerosis from arteriosclerosis. Hauswirth and Eisenberg<sup>11</sup> regarded phlebosclerosis as a disseminated process found frequently in association with peptic ulcers.

In portal cirrhosis, in which the dynamic factor of increased intra-venous pressure is present, Simmonds<sup>10</sup> recorded sclerosis of the portal vein. Lossen<sup>12</sup> reported sclerosis of the portal, splenic, mesenteric and coronary veins in portal cirrhosis with hepatosplenomegaly. McIndoe<sup>13</sup> demonstrated sclerosis of both the portal system and the hepatic veins in portal cirrhosis.

Carrel<sup>14</sup> found scars in arteriovenous anastomoses, especially at points where the caliber of a vessel changed. The vein reacted to the arterial pressure by increase in thickness and strength of its wall, observed histologically to be the result of hypertrophy. Reid<sup>15</sup> was of the opinion that the altered intravascular pressure was responsible for the final atrophy of the artery and hypertrophy of the vein. Callander<sup>16</sup> noted increasing dilatation of the veins in arteriovenous fistulas, due

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5 Kaya, R. *Virchows Arch f path Anat* **189** 466, 1907.

6 Schilling, W. *Virchows Arch f path Anat* **262** 658, 1926.

7 Waaler, E. *Am J Path* **13** 855, 1937.

8 Allen, E. V., and Page, I. H. *Deutsches Arch f klin Med* **168** 193, 1930.

9 Sack, G. *Virchows Arch f path Anat* **112** 403, 1888.

10 Simmonds, M. *Virchows Arch f path Anat* **207** 360, 1912.

11 Hauswirth, L., and Eisenberg, A. *Arch Path* **11** 858, 1931.

12 Lossen, J. *Mitt a d Grenzgeb d Med u Chir* **13** 752, 1904.

13 McIndoe, A. H. *Arch Path* **5** 23, 1928.

14 Carrel, A. *Technique and Remote Results of Vascular Anastomoses*, in *Studies from the Rockefeller Institute for Medical Research*, 1912, vol 15, no 27.

15 Reid, M. R. *Am J Surg* **14** 17, 1931.

16 Callander, C. L. *Johns Hopkins Hosp Rep* **19** 259, 1920.



in his opinion to the rise of venous pressure from passage of arterial blood through the sac Kaufmann<sup>17</sup> described sclerosis of the walls of varicose veins Benda<sup>18</sup> reported similar changes and observed intimal thickening, deposition of fibrous tissue, muscular atrophy, new formation of elastic tissue and connective tissue infiltration within and between the muscle bundles of the media of veins

#### ANALYSIS OF MATERIAL

Before proceeding with the presentation of our findings it is advisable to discuss briefly the structure of the normal superior vena cava As Franklin<sup>19</sup> ably pointed out in his excellent monograph on the veins, no true understanding of the histology of these vessels can be obtained without a consideration of their function and location

The superior vena cava, owing to its large blood flow, its low venous pressure and the support afforded by the mediastinal structures, is a large thin-walled vein Its endothelial lining merges imperceptibly with that of the right auricle The intima is narrow and rests on a narrow network of interlacing elastic fibers Collagen occurs in spirals in the superior vena cava and in vessels that vary in length The well developed circular muscle in the media and adventitia of the inferior vena cava and in the veins of the lower extremities as compared with the thinner-walled veins of the upper parts of the body, which are poor in muscle fibers and collagen, is definitely related to greater internal pressure in the former vessels The function of the circular elastic fibers is to accommodate sudden increases in content The longitudinal elastic fibers prevent collapse of vessels of low internal pressure Sparsely scattered individual smooth muscle fibers are found in this layer The rather wide and loosely constructed adventitial layer surrounds the media, and in the outer portions of this layer are seen bundles of cardiac muscle These fibers, which no doubt give support to the vessel wall, ascend in a spiral direction for variable distances along the vein

The superior venae cavae from 21 persons with chronic congestive heart failure were examined post mortem As control material the venae cavae from patients who during life and at necropsy showed no evidence of cardiac insufficiency or cardiac disease were used

Tissue was taken at a point within 3 cm of the junction of the superior vena cava with the right auricle The gross appearance of the wall was noted, and

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17 Kaufmann, E Lehrbuch der speziellen pathologischen Anatomie, Berlin, W de Gruyter & Co, 1922

18 Benda, C, in Henke, F, and Lubarsch, O Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1924, vol 2

19 Franklin, K J A Monograph on Veins, Springfield, Ill, Charles C Thomas, Publisher, 1937

the material was fixed according to standard technics. Histologic sections 6 microns thick were stained with hematoxylin and eosin and with Verhoeff's elastic tissue stain and Van Gieson's stain. The sections were then examined without knowledge of the clinical diagnoses, in order to remove any prejudicial factor.

Of the 21 patients with chronic congestive heart failure, 14 were males and 7 were females, the ages ranged from 47 to 70 years. No attempt was made to select material from those in whom congestive heart failure was due to any particular etiologic agent. The causes of congestive heart failure in these patients were hypertensive cardiovascular disease in 15, arteriosclerotic cardiovascular disease in 2, rheumatic cardiovascular disease in 2 and cor pulmonale cardiovascular disease in 2. The duration and severity of the congestive heart failure in 21 of these patients are given in the table.

The superior venae cavae from 2 persons with hypertensive cardiovascular disease and 1 person with subacute bacterial endocarditis were examined also. Of the former one died following coronary thrombosis, the other died of a cerebral insult. None of these suffered from congestive heart failure.

The venae cavae from 8 persons not dying of cardiac diseases were also examined. The diagnoses in this group were chronic pulmonary tuberculosis in 5, status asthmaticus in 1, generalized sarcomatosis in 1 and Kaposi cell sarcoma in 1.

*Duration and Severity of Congestive Heart Failure in Twenty-One Cases*

Duration, Yr.	Cases	Cases of Given Type		Duration, Yr.	Cases	Cases of Given Type	
		Moderately Severe	Severe			Moderately Severe	Severe
1	3	1	2	5-10	4	0	4
1-2	4	2	2	10-16	2	1	1
2-5	8	6	2				

#### DATA

*Superior Venae Cavae from Group with Chronic Congestive Heart Failure*—Macroscopic changes were found in only 2 of the 21 superior venae cavae examined. One of these was from a person with moderate congestive failure of eight months' duration, the other was from a person with multiple myocardial infarction and severe failure of seven years' duration. The alterations in each vein consisted of slightly raised yellowish plaques, varying in size from 0.5 to 2 cm. in diameter. Microscopically, these areas were composed of definite accumulations of loose connective tissue separated by rather wide tissue spaces, which in the latter vein were infiltrated by numerous small round cells.

Microscopic intimal alterations were observed in 18 of the 21 veins. In 4 there was slight thickening of this layer, while in the remaining 14 moderate to marked thickening was present. The thickening was largely the result of an increase in collagenous connective tissue. In 6 veins smooth muscle elements were also found in the thickened intima. In addition to the plaques described, a microscopic plaque composed of an acellular homogeneous hyaline substance was found in a vein

from a patient with hypertensive cardiovascular disease and diabetes who had suffered from progressively severe congestive heart failure for two years. Finally, a small connective tissue scar was found in a vein from a patient who had chronic rheumatic heart disease with severe congestive failure of four years' duration. The scar was infiltrated with small round cells, but no Aschoff bodies were found. Whether this scar represented the terminal healed phase of an Aschoff body or was

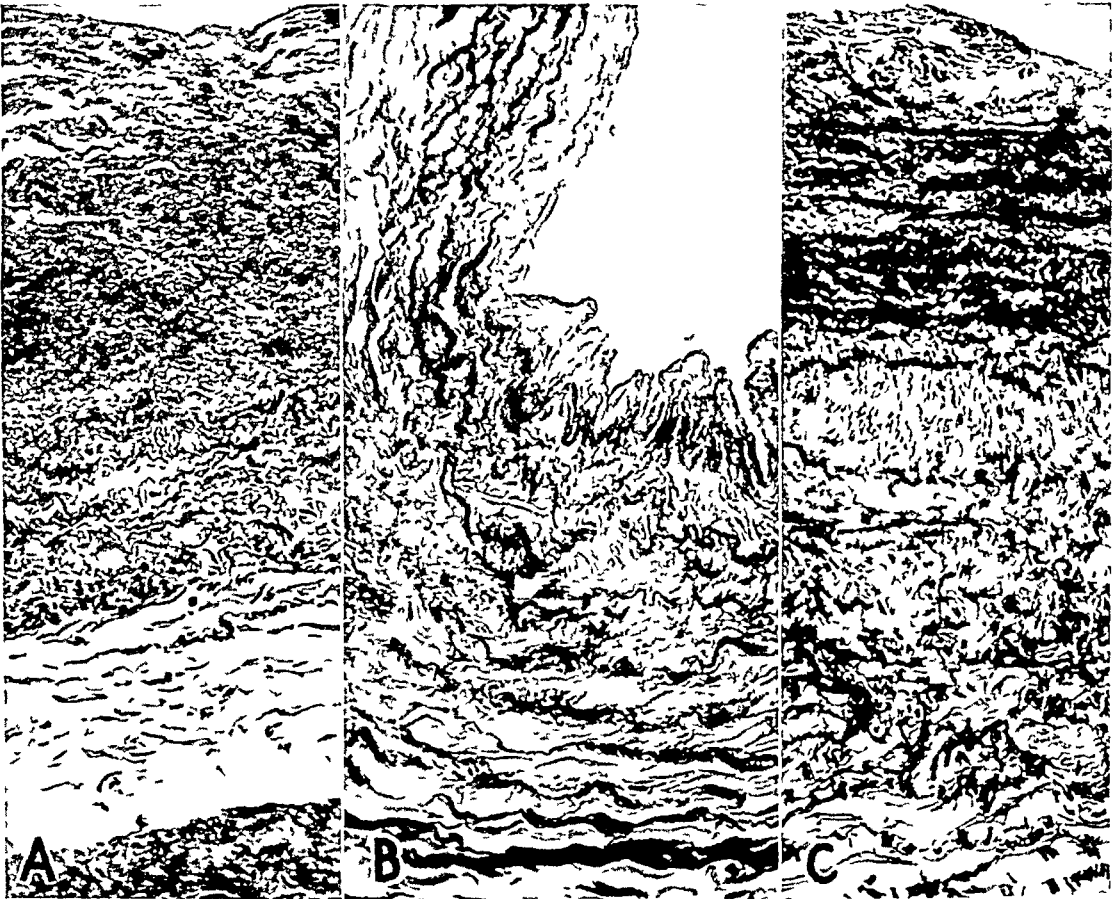


Fig 1—*A*, marked intimal and medial hypertrophy in the superior vena cava of a man of 64 years with hypertensive heart disease and moderate congestive heart failure of eight months' duration,  $\times 100$ . *B*, thickening of the intima, splitting and reduplication of the elastica and hypertrophy of the media due to dense deposits of collagen and hyperplasia of the smooth muscle cells in the superior vena cava of a woman of 73 years with hypertensive heart disease and known congestive heart failure of three months' duration,  $\times 100$ . *C*, raised intimal plaque with round cell infiltration and medial hypertrophy in the superior vena cava of a man of 57 years with severe hypertensive heart failure of seven years' duration, cardiac muscle is present in the adventitia,  $\times 200$ .

a sclerotic patch due to chronic congestive heart failure cannot be stated. Alterations in the internal elastic layer were observed in 10 veins. These changes consisted chiefly of thickening, splitting and reduplication of

the elastic fibers, so that in some cases as many as three separate and distinct layers could be discerned

Hypertrophy of the media due to the presence of numerous smooth muscle cells, hypertrophy of the smooth muscle cells and hypertrophy of the collagenous fibers was the most constant observation in the superior venae cavae in cases of congestive heart failure. Thickening

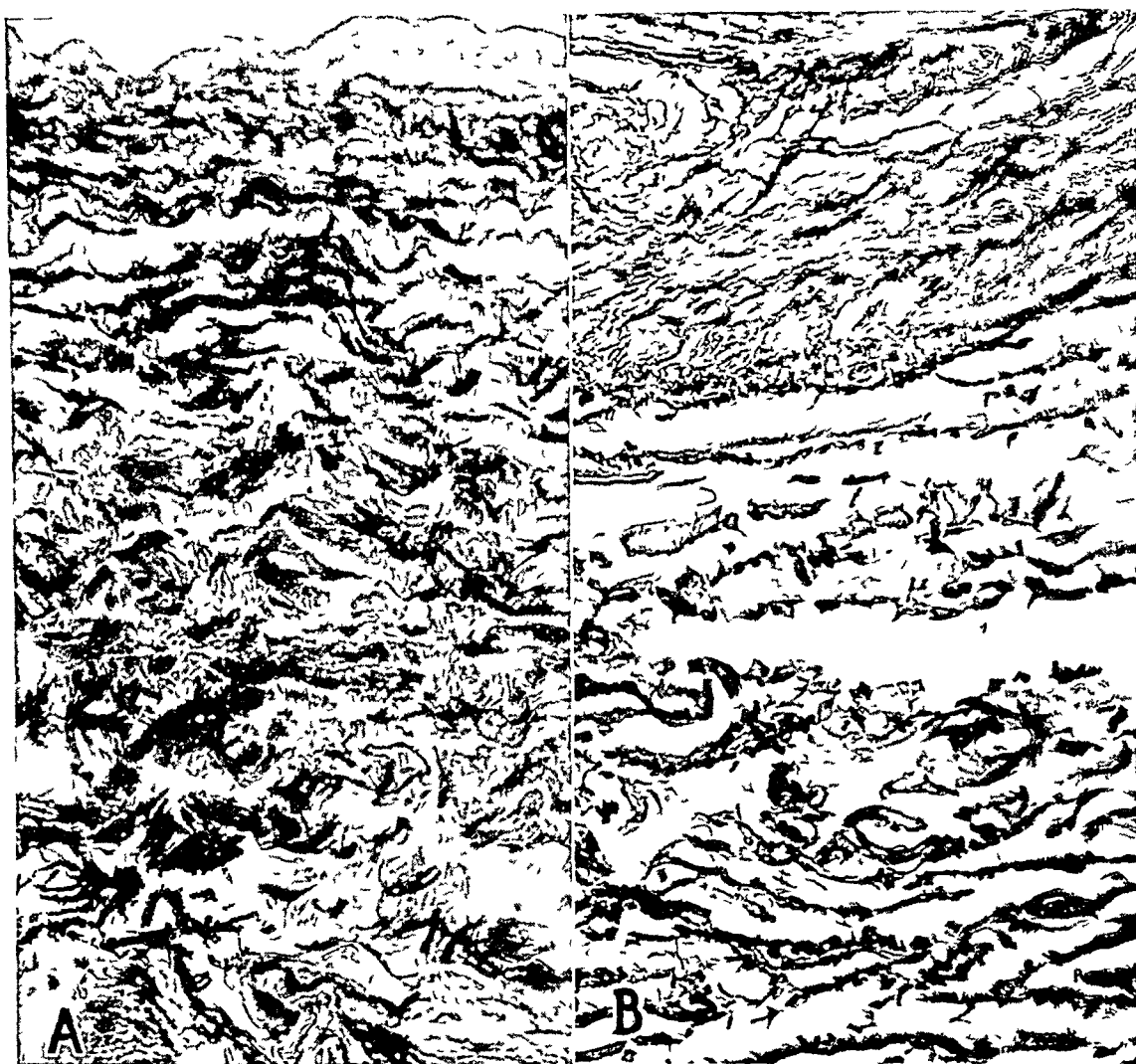


Fig 2—*A*, medial hypertrophy, with a large amount of collagen and large numbers of fragmented smooth muscle cells, and ingrowth of elastica in the superior vena cava of a man of 51 years with severe hypertensive heart failure of six years' duration,  $\times 240$  *B*, marked medial hypertrophy due to marked hyperplasia of smooth muscle cells and collagen in the superior vena cava of a man 61 years old with diabetes and hypertension and progressively severe congestive heart failure of two years' duration,  $\times 200$

of the media was marked in 11 instances, moderate in 6 and absent in the remaining 4. In some cases collagenous fibers were arranged in an irregularly fragmented bizarre fashion suggesting old scars. Smooth

muscle fibers were found in the media of 19 of the sections studied. In 12, the muscle cells were found in abundance, while in the other 7 they were scattered and few.

In 17 of the 21 veins the increase in the muscle fibers was coincident with hypertrophy of the media. Although in the remaining 4 veins there was no increase in thickness of the media, other significant changes were present. Marked intimal changes were found in 3, and scattered or numerous smooth muscle cells were present in 2 others. Combined medial and intimal changes occurred in 14, combined medial and elastica alterations occurred in 8. Intimal thickening and elastica changes were combined in 9 veins and in only 7 were alterations in all three layers seen. It is noteworthy that no vein in this group demonstrated changes in less than two layers.

*Superior Venae Cavae from Group Not Having Congestive Heart Failure*—Macroscopic changes were found in none. Slight thickening of the intima was noted in the superior venae cavae from a patient with hypertension, a patient with rheumatic heart disease and subacute bacterial endocarditis and a patient with pulmonary tuberculosis and hypertrophy of the right ventricle. Moderate thickening of the intima occurred in but one vein in this group, that of a patient with hypertension. Splitting or reduplication of the elastica was found in the veins of 2 patients with pulmonary tuberculosis, 1 of whom had hypertrophy of the right ventricle. The media was moderately hypertrophied in 2 veins and markedly hypertrophied in 2 others. The latter were, respectively, from a patient with hypertension who died in status asthmaticus of acute failure of the right side of the heart and from a patient with subacute bacterial endocarditis engrafted on rheumatic mitral disease who at necropsy was observed to have chronic passive congestion of the liver and spleen. The vein of the latter showed also a round cell infiltration, so that it is possible that the findings represent the end stage of an Aschoff body in a vein.

Muscle fibers were observed in the media in 5 veins. In 3 veins (1 from a patient with pulmonary tuberculosis and hypertrophy and dilatation of the right side of the heart and 2 from patients with hypertension) the muscle cells were few. A large number of muscle cells was present in the media of a vein from a rheumatic patient with chronic passive congestion of the liver and spleen and in that of a tuberculous patient, both patients showed hypertrophy and dilatation of the right side of the heart.

In summary, in this group, involvement of the superior vena cava was minimal, none showing significant hypertrophy of the intima or media. In no single instance of the 11 were changes found in all the layers of the vein. Furthermore, in those in which definite hypertrophy

or sclerosis of the superior vena cava was noted there was increased tension in the right side of the heart due either to pulmonary tuberculosis and fibrosis, hypertension or mitral stenosis. The alterations were less marked and occurred as isolated phenomena in some of the superior venae cavae of the group not in congestive failure. It is striking that even in those instances in which there was increased tension in the right side of the heart the degree of hypertrophy and sclerosis of the superior vena cava never equaled in extent and severity that seen in cases of chronic congestive heart failure.

#### COMMENT

Persistently increased tension in the vascular tree is generally accepted as the prime cause of vascular sclerosis. The order of sclerosis of the circulation may be listed as (1) arteriosclerosis of the greater circulation, (2) arteriosclerosis of the lesser circulation, (3) sclerosis of the venous system and (4) localized arteriosclerosis or phlebosclerosis. This order corresponds to the frequency of increased intravascular tension of the different parts of the circulation. In a given case it is not unusual, however, to see isolated phlebosclerosis in association with varicose veins of one leg or marked pulmonary arteriosclerosis or phlebosclerosis associated with mitral stenosis in a young person though the greater circulation is essentially intact.

It is well known that the tension in the superior vena cava is usually at or below zero and is raised above zero only in exceptional circumstances. In conditions in which the venous pressure in the liver is increased, as in portal cirrhosis, sclerosis of the hepatic veins is a common finding. In congestive heart failure the liver acts as a reservoir for the overloaded lungs. Consequently the hepatic veins and the superior and inferior venae cavae are chronically distended. As in sclerosis of the pulmonary artery and vein in pulmonary hypertension from mitral stenosis, the conditions in which there is chronic increase of tension in the hepatic veins are associated with phlebosclerosis of a high degree. The occurrence of phlebosclerosis at such sites of increased venous pressure favors the view that venous sclerosis also is due to increased tension in the vessel wall. Though phlebosclerosis is less common than arteriosclerosis, it is significant that in conditions in which venous pressure is increased over a long time phlebosclerosis occurs with great constancy.

In our material, though the degree of sclerosis of the superior vena cava paralleled roughly the degree of increased tension in the vessel wall, no exact correlation could be observed between either the severity or the duration of congestive heart failure and the degree of phlebo-

sclerosis In some cases mild congestive failure or failure of short duration was observed with severe phlebosclerosis, while in others in which failure of either severe or long duration was noted, phlebosclerosis was relatively slight The same fact holds, however, for hypertension of the greater or lesser circulation and arteriosclerosis In these conditions there is also no exact correlation between vascular damage and the duration of increased tension In another study<sup>2</sup> no exact correlation was possible between the degree of failure of the right side of the heart and the degree of associated sclerosis of the hepatic veins and inferior vena cava It is possible that this disparity is due to varying degrees of anoxemia or to metabolic causes

There is great similarity between arteriosclerosis and phlebosclerosis both in pathogenesis and in morbid anatomy In arteriovenous aneurysm and fistula and in experimental arteriovenous anastomosis it may be shown that when the vein is put under increased tension or required to do increased work hypertrophy of its wall occurs Eventually, atrophy of its wall occurs from nutritional impairment In our findings the development of hypertrophy of the intima and of the smooth muscle of the media followed by atrophy supports the view of a similar mechanism The view that the changes observed were due to increased tension from congestive heart failure is further supported by the fact that in the cases in which there was no failure the alterations were less marked or were isolated phenomena It is significant that even in cases in which there was increased tension in the right side of the heart without failure the hypertrophy and sclerosis of the superior vena cava never equaled in extent or severity such impairment in cases of congestive heart failure Since the degree of hypertrophy and sclerosis of the superior vena cava varied roughly in relation to the degree of increased tension of the right side of the heart, both the hypertrophy of the wall of the vein and its ultimate degeneration in congestive heart failure of long duration may be looked on as a response to increased tension

#### SUMMARY

The superior venae cavae of 21 persons showing chronic congestive heart failure were studied and compared with those of a group showing hypertrophy of the right side of the heart without failure and with those of another group in whom there was no cardiac lesion at all

In persons with chronic congestive heart failure associated with increased tension in the right side of the heart and in the superior vena cava, sclerosis of the superior vena cava is a common finding

Histologically, the sclerotic process in the superior vena cava is characterized by hypertrophy of all the coats of the vein, most marked in the muscular layer of the media These alterations are thickening

and scarring of the intima, splitting and reduplication of the internal elastic membrane and widening of the media with hypertrophy of the muscle cells and increase of collagen. Eventually, from increased tension and impairment of nutrition, fragmentation and replacement of muscle fibers occur.

Involvement of the superior venae cavae from persons not having congestive heart failure was slight and infrequent, and in not a single vein were all the coats of the vessel involved. Medial hypertrophy, which was so constant and marked in the superior venae cavae of the group who died in congestive heart failure, was an infrequent occurrence.

Phlebosclerosis and arteriosclerosis are similar in morbid anatomy and pathogenesis. The pathogenesis of sclerosis of the superior vena cava appears to be in prolonged increase of intravascular pressure.



# EFFECT OF EXPERIMENTAL NEUTROPENIA ON THE HEALING OF WOUNDS\*

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In a previous study<sup>1</sup> on a serum toxic to polymorphonuclear neutrophil leukocytes, it was shown that these cells could be greatly diminished in number or totally eliminated from the peripheral blood of guinea pigs. Repeated daily doses of increasing amounts of the anti-serum maintained this condition for several days, after which a gradual increase in the number of the neutrophils occurred.

This finding led to speculation on the effect such neutropenia would have on the healing of wounds. Neutrophils are seen in large numbers about any area of injury to tissue, which has led some observers to believe that they play a part in the healing process. The object of our study was to test this hypothesis.

## METHODS

Guinea pigs were used. Under aseptic precautions an incision was made through all layers of the abdominal wall and the edges of the wound were reapproximated using 000 plain catgut for the peritoneum and fascia recta and silk for the skin. Visceral wounds were tried, particularly wounds in the stomach, but were not as satisfactory as wounds in the abdominal wall. In one series the incisions were contaminated with *Staphylococcus aureus* in order that we might observe healing in the presence of infection.

After the animals were killed, an effort was made to determine the degree of healing with a machine testing tensile strength, but the wounds separated so easily that often no record could be obtained, hence these results were unreliable. The wounds were then ruptured with air pressure by slowly inflating the peritoneal cavity. A mercury manometer in the circuit gave the tension required for disruption. Microscopic sections were made through all layers of the wounds.

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\* Aided in part by a grant from the Committee on Scientific Research of the American Medical Association.

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1 Chew, W B, Stephens, D J, and Lawrence, J S. *J Immunol* **30** 301, 1936.

The antileukocytic serum was prepared, sterilized and standardized by the methods described<sup>1</sup> It had been found that a dose of 0.5 cc., given intraperitoneally, was sufficient to produce neutropenia, so this amount was given as the initial dose to the majority of the animals. The dose was increased daily by amounts sufficient to keep the number of the neutrophils at a low level. In approximately one-half the animals, the initial injection of the antiserum was on the morning of operation, but in the majority of the other animals the initial injection was on the day preceding operation. In 2 instances, the administration of antiserum was begun two days before operation. In every instance, following the injection of the initial dose injections were given daily.

Sufficient white blood cell counts, total and differential, were made prior to operation to establish the normal values for each animal. Following operation, daily total and differential counts were made until the animal was put to death. The table gives the actual number of polymorphonuclear neutrophils prior to

*Neutrophils Per Cubic Millimeter Before and After Operation*

Guinea Pig	Before Operation*	Days After Operation						Comment
		1	2	3	4	5	6	
1	2,277	30	11					Injections begun day before operation
2	7,337	62	13	0				Injections begun day before operation
3	7,128	450	0	0				Injections begun day of operation
4	4,945	216	0	39				Injections begun day of operation
5	10,807	700	243	81	61			Injections begun day before operation
6	2,756	0	0	0	35			Injections begun day of operation
7	4,644	21	0	0	0			Injections begun day of operation
8	1,131	0	108	192	60			Injections begun day of operation
9	6,070	765	78	28	360	1,193		Injections begun 2 days before operation
10	2,160	0	0	0	0	72		Injections begun day of operation
11	6,020	5,986	333	0	19	0		Injections begun day of operation
12	2,748	138	0	400	0	100	845	Injections begun day of operation
13	3,524	30	16	21	196	122	273	Injections begun 2 days before operation
14	2,888	82	47	246	51	61		Injections begun day of operation
15	7,285	234	140	168	451			Injections begun day before operation (infected)
16	2,484	0	0					Injections begun day before operation
17	6,165	0						Injections begun day before operation
18	2,496	0						Injections begun day before operation

\* Figures in this column represent those obtained for each animal in the last determination prior to the injection of antiserum.

operation and on each day after operation. While there are a few exceptions, it will be noted that the level of neutrophils was kept very low following operation. Such neutropenia is difficult to maintain longer than six days owing to the fact that the amount of antiserum required to produce it increases progressively with each day. Accordingly, observations were not carried beyond this length of time. It is felt that this period is sufficient for observation of the essentials of the healing process but of course not for following it to its completion.<sup>2</sup>

## RESULTS

Forty-two guinea pigs were used, half of these were given antiserum and the remainder were used for controls.

One series of 34 animals, 17 of which had been rendered neutropenic, were operated on under aseptic precautions. The gross appearance of the wounds of the treated guinea pigs did not differ from those of the

untreated animals The tensile strength as measured by the air pressure required to disrupt the wounds varied over wide limits The average disrupting force was 132 mm of mercury for the neutropenic and

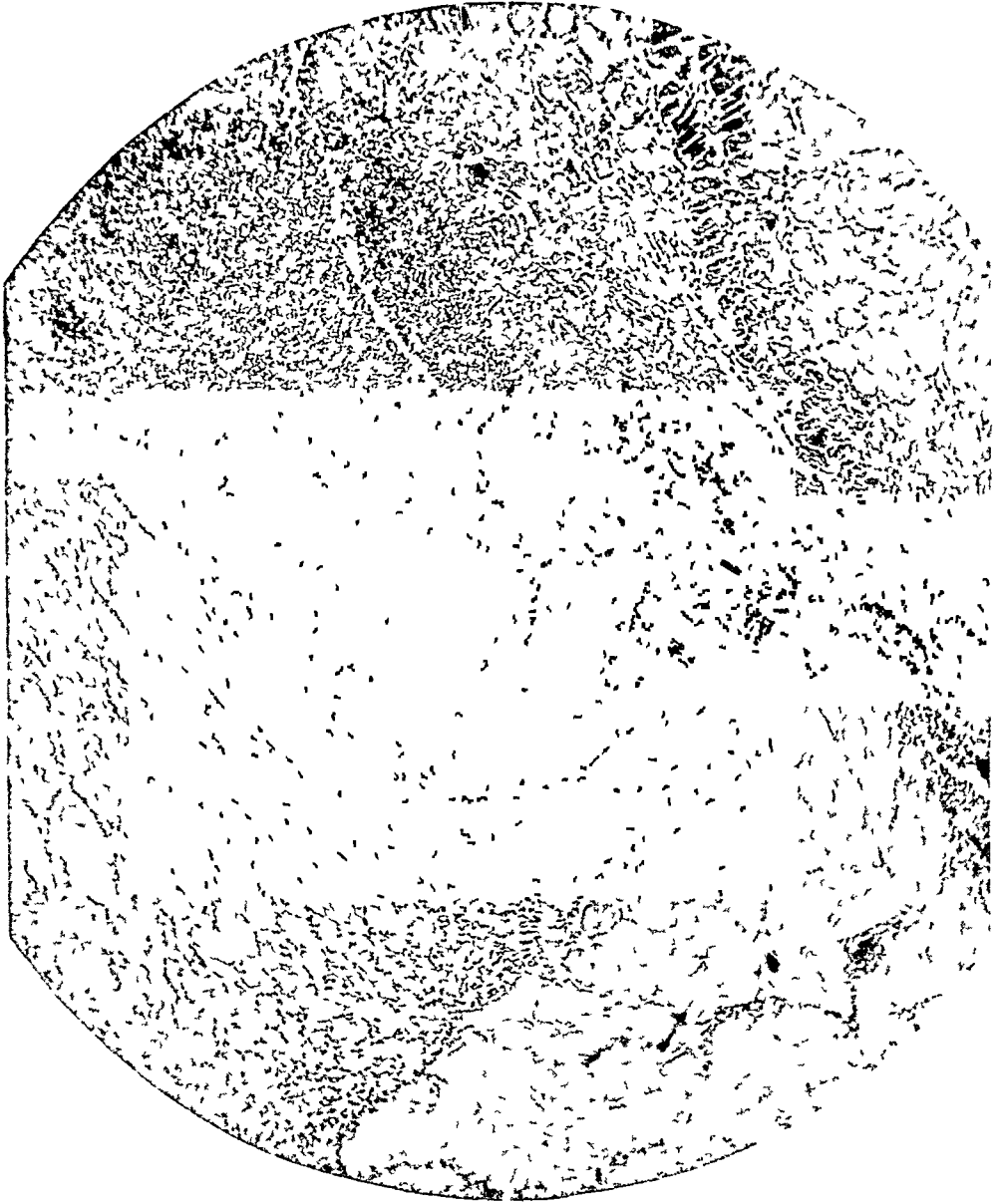


Fig 1—Photomicrograph of an infected wound in a normal control guinea pig Note the accumulation of large numbers of polymorphonuclear neutrophils

196 mm for the control animals The difference was not considered significant in this small group

In judging repair, the greatest reliance was placed on the histologic appearance of the healing process This was quite different in the two

groups, the striking contrast lying in the reduction or absence of neutrophils in the treated animals which nevertheless, had as much fibroblastic proliferation as the controls. Fibroplasia and repair occurred



Fig 2—Photomicrograph of an infected wound in a neutropenic guinea pig. No polymorphonuclear neutrophils are present.

equally well in the presence or in the absence of polymorphonuclear leukocytes in the wounds.

A few of both the treated and the control animals showed occasional bacteria in the tissues, though there was no gross evidence of infection.

in any of the wounds in these animals. The appearance differed here also, for in the neutropenic guinea pigs there were few if any leukocytes about the bacteria, while the controls showed the usual defense reaction of aggregations of polymorphonuclear cells. This led to a trial of deliberate infection of the wounds with *Staph aureus* at the time of operation in 8 guinea pigs. These neutropenic animals had little defense against this insult. With but a single exception, they presented diffuse cellulitis of the abdominal wall, injection of the peritoneum and hemorrhagic discharge from the wounds. The microscopic appearance of the tissue was not unlike postmortem degeneration with necrosis of tissue—many bacteria but few cells. The control animals responded in the usual way, with localization of the infection to form an abscess in which were found masses of leukocytes.

#### COMMENT

It appears from the results stated that the polymorphonuclear neutrophil plays a part in the repair of wounds only so far as it helps to combat suppuration. The essential feature of repair is fibroplasia, and this process is apparently independent of the neutrophilic leukocyte. This indirectly supports the concept that the fibroblasts may originate by metaplasia from the lymphocytes or macrophages.

Some histologists have thought that the polymorphonuclear leukocytes liquefied the fibrin in the wound and so paved the way for ingrowth of fibroblasts. In this way they were considered an essential factor in the reparative process. This is apparently incorrect, for fibroplasia progressed normally when there were few if any neutrophils in the wounds.

It is worthy of comment that guinea pigs with so few neutrophils as reported in this paper did not contract gross infection during the period of observation. That they were more susceptible to infection than normal animals was shown by the response to virulent cultures of *Staph aureus* put into the wounds. However, with aseptic technic the wounds did not get infected. Further, we have never noted oral, perianal or other types of spontaneous infection in guinea pigs kept neutropenic for varying periods. This indicates that the neutropenic guinea pig is less susceptible to infection than the neutropenic human subject.

#### SUMMARY

Healing of aseptic wounds in guinea pigs has been shown to be unaffected by the presence or the absence of neutrophils.

In neutropenic guinea pigs that have septic wounds there is marked inability to cope with the healing process.

## SO-CALLED BILIARY CIRRHOSIS

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AND

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ROCHESTER, MINN

Since the beginning of knowledge of hepatic cirrhosis, the subject has been in turmoil Major<sup>1</sup> gave credit to John Brown for being the first to describe a cirrhotic liver, in 1685 Brown<sup>2</sup> at necropsy on a patient with marked ascites noted the "glandulous appearance" of the liver He illustrated this description with a picture of a coarsely nodular liver Laennec<sup>3</sup> introduced the term "cirrhosis of the liver" in 1826 It is of particular interest that he recognized that "this type of growth belongs to the group of those which are confused under the name of Scirrhus I believe we ought to designate it with the name of cirrhosis, because of its color" In addition he stressed the fact that there was progressive atrophy and observed that similar changes occurred in other organs Webster's "New International Dictionary" derives the term "cirrhosis" from the Greek word *κνίθος*, meaning orange colored It is not strange, but it is confusing and unfortunate, that in general "cirrhosis" has become almost synonymous with "fibrosis" Kaufmann<sup>4</sup> observed that "cirrhosis," meaning yellow, was a name applied first to the contracted liver and that later it was applied generally to organ-shrinking processes which are accompanied by more or less formation of connective tissue, such as cirrhosis of the lung or of the kidney, and that the color was then ignored

Even as the original description of hepatic cirrhosis offers little help in defining the disease, so the current conceptions of the disease differ too widely to be of any help Mallory<sup>5</sup> applied the term "cir-

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\* Fellow in Medicine of the Mayo Foundation at the time this work was done

1 Major, R H Classic Descriptions of Disease, Springfield, Ill, Charles C Thomas, Publisher, 1932, pp 597-602

2 Brown, J Phil Tr Roy Soc, London 3 248, 1685

3 Laennec, R T H Traite de l'auscultation mediate et des maladies des poumons et du cœur, ed 2, Paris, J S Chaude, 1826, vol 2, pp 187-197

4 Kaufmann, E Pathology for Students and Practitioners, translated by S P Reimann, Philadelphia, P Blakiston's Son & Co, 1929, pp 921-928 and 954-955

5 Mallory, F B Bull Johns Hopkins Hosp 22 69, 1911

rhosis" "to all sclerosed conditions of the liver, whether progressive or not, in which destruction of liver cells is associated with real or apparent increase of connective tissue" Emphasis is clearly laid on parenchymal destruction and fibrosis

Rossle<sup>6</sup> stated that "it can be said that liver cirrhosis is due to three main factors destruction of liver tissue, scar tissue formation and compensatory hyperplasia, or regeneration, respectively, and therefore in spite of various etiologic factors and different appearances it might be considered a disease entity" The prominence of these individual features varies in the different types of "cirrhosis" In his discussion he stressed the fibrosis and parenchymal damage He recognized that parenchymal regeneration may occur but did not require its presence Eppinger<sup>7</sup> quoted Rossle frequently and apparently endorsed his opinion

Kaufmann,<sup>4</sup> speaking of the common atrophic cirrhosis of the liver, stated that "this form of chronic hepatitis depends on a marked connective tissue development with destruction of considerable liver tissue" He further added that parenchymal hyperplasia, even nodular or adenomatous in character, may occur in some cirrhoses

Bell<sup>8</sup> stated that "cirrhosis (of the liver) is a very slowly progressing degenerative and reparative process, apparently inflammatory in nature, involving the entire organ and characterized by a definite increase of the portal connective tissue with or without an increase of the intralobular connective tissue" In his discussion of the microscopic features of portal cirrhosis he further stated that "the proliferation of these structures (the newly formed bile ducts) is at times so extensive as to produce rounded, grossly visible, pale nodules, the so-called adenomata" He gave credit to both the interlobular bile ducts and the parenchymal remnants for the formation of the parenchymal nodules

MacCallum<sup>9</sup> defined cirrhosis of the liver as "a term applied to an extensive diffuse scarring of the liver which has followed the destruction of much of the liver substance It is regularly accompanied by widespread regeneration of the functional liver tissue, usually sufficient to prevent the appearance of any signs of hepatic insufficiency" In

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6 Rossle, R Entzündungen der Leber, in Henke, F, and Lubarsch, O Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1930, vol 5, no 1, pp 284, 286-313, 376-405 and 429-452

7 Eppinger, H Die Leberkrankheiten Allgemeine und spezielle Pathologie und Therapie der Leber, Berlin, Julius Springer, 1937, pp 121, 148 and 571-598

8 Bell, E T A Text-Book of Pathology, ed 3, Philadelphia Lea & Febiger, 1938, pp 688-695

9 MacCallum, W G A Text-Book of Pathology, ed 6, Philadelphia, W B Saunders Company, 1936, pp 304-316

his discussion of the microscopic features of diffuse nodular cirrhosis (Laennec's cirrhosis) he stressed the disruption of the lobular architecture by nodular hyperplasia. He further stated that "although many conflicting views have been held, it seems clear that the injurious agent effects the destruction of the liver cells in the first instance, and that the scarring and the hyperplasia of the epithelial remnants are reparatory processes."

From these brief references to several authorities it is clear that a definition of hepatic cirrhosis acceptable to all is difficult to find. There is general agreement that parenchymal destruction and scarring must be present. There is less agreement on the importance of parenchymal repair. When only parenchymal destruction and scarring are required by definition of hepatic cirrhosis, it is difficult to rule out certain conditions recognized as not generally deserving of the designation "hepatic cirrhosis."

Many agents often produce obvious parenchymal destruction and at least an apparent increase in connective tissue. Of the chemical agents, cinchophen may be mentioned, and of the physiologic disturbances, severe chronic passive congestion, hyperthyroidism and even biliary obstruction may be mentioned. However, these factors are not regarded as frequent causes of cirrhosis of the liver. The ill defined yellow atrophy of the liver is not and should not be classified ordinarily as hepatic cirrhosis, although parenchymal destruction and an apparent increase in connective tissue are commonly present.

In brief, a definition including simply parenchymal destruction and scarring seems too broad. Such a definition apparently includes lesions which it does not seem justifiable to classify under hepatic cirrhosis. Hence, for the purpose of this study, hepatic cirrhosis is defined as including parenchymal degeneration, fibrosis and nodular parenchymal repair.

In this investigation the primary interest lies, not in the general subject of hepatic cirrhosis and the multiple subtypes of this disease, but in the subject of so-called biliary cirrhosis. This term, fostered by the French, has gained some recognition in Germany and is frequently used by pathologists and clinicians on this continent. The features of biliary cirrhosis will be presented subsequently, but it may be said here that clearly this type of disease of the liver as usually described is to be excluded according to the foregoing definition of hepatic cirrhosis.

The definition of hepatic cirrhosis given seems in accord with the views of MacCallum.<sup>9</sup> However, in his discussion of obstructive biliary "cirrhosis" he did not mention parenchymal repair. One can hardly say that he suggested it may occur when he stated "Apparently in



the cases of longest duration (prolonged biliary obstruction) a great deal more distortion of the liver may occur, but it does not approach that seen in the previous type (diffuse nodular cirrhosis) "

## LITERATURE

General interest in the possibility of hepatic cirrhosis following biliary obstruction received its main impetus from the clinical and experimental observations of Charcot and Gombault<sup>10</sup> and Charcot<sup>11</sup>. They postulated cirrhosis of biliary origin in contradistinction to that of portal origin. However, the foundations for their postulate had been laid by the reports of Jones,<sup>12</sup> Wyss,<sup>13</sup> Leyden,<sup>14</sup> Mayer,<sup>14</sup> Green,<sup>15</sup> and Legg<sup>16</sup>. The earlier cases were reviewed by Mangelsdorf<sup>17</sup> and Ford,<sup>18</sup> but since then a vast literature on the subject has accumulated.

Pure biliary stasis has been regarded as sufficient to produce "biliary cirrhosis" by Quincke,<sup>19</sup> Richardson,<sup>20</sup> Ogata,<sup>21</sup> Lieber and Stewart,<sup>22</sup> MacMahon and Mallory<sup>23</sup> and others. Litten,<sup>24</sup> Nasse,<sup>25</sup> Ford,<sup>18</sup> Rossle<sup>6</sup> and others have emphasized the importance of infection or inflammation within the biliary tract in the production of this disease. Beloussow,<sup>26</sup> Rolleston,<sup>27</sup> and Eppinger<sup>7</sup> were not convinced that biliary obstruction resulted in cirrhosis in man. Senator,<sup>28</sup> Ford<sup>18</sup> and Kauf-

10 Charcot, J. M., and Gombault, A. *Arch de physiol norm et path* **3** 272, 1876

11 Charcot, J. M. *Leçons sur les maladies du foie, des voies biliaires et des reins*, Paris, aux bureaux du *Progres medical*, 1877, pp 160-166 and 205-218

12 Jones, H. *Tr Path Soc London* **5** 146, 1854

13 Wyss, O. *Virchows Arch f path Anat* **35** 553, 1866

14 Cited by Legg<sup>16</sup>

15 Green, T. H. *Tr Path Soc London* **23** 133, 1872

16 Legg, J. W. *St Barth Hosp Rep* **9** 161, 1873, *Tr Path Soc London* **25** 133 and 155, 1874

17 Mangelsdorf, J. *Deutsches Arch f klin Med* **31** 522, 1882

18 Ford, W. W. *Am J M Sc* **121** 60, 1901

19 Quincke, H. *Diseases of the Liver, Pancreas, and Suprarenal Glands*, in Nothnagel, H. *Encyclopedia of Practical Medicine*, translated by A. Stengel, Philadelphia, W. B. Saunders Company, 1903, pp 431 and 727-729

20 Richardson, M. L. *J Exper Med* **14** 401, 1911

21 Ogata, T. *Beitr z path Anat u z allg Path* **55** 236, 1913

22 Lieber, M. M., and Stewart, H. L. *Arch Path* **17** 362, 1934

23 MacMahon, H. E., and Mallory, F. B. *Am J Path* **5** 645, 1929

24 Litten, M. *Charite-Ann* **5** 153, 1880

25 Nasse. *Arch f klin Chir* **48** 885, 1894

26 Beloussow, P. N. *Arch f exper Path u Pharmakol* **14** 200, 1881

27 Rolleston, H. D. *Diseases of the Liver, Gall-Bladder and Bile-Ducts*, Philadelphia, W. B. Saunders Company, 1905, pp 326-332

28 Senator, H. *Berl klin Wchnschr* **30** 1233, 1893

man<sup>29</sup> recognized obstructive "biliary cirrhosis" and stated that biliary obstruction in man may lead to later hepatic shrinkage or atrophy. Janowski<sup>30</sup> held that death would occur before hepatic contraction resulted. Judd and Counseller,<sup>31</sup> Greene and his co-workers<sup>32</sup> and Weir and Snell<sup>33</sup> found what they called obstructive biliary cirrhosis was more frequently associated with benign than with neoplastic obstructive biliary lesions.

Numerous investigators have credited the experimental ligation of the extrahepatic bile ducts with the production of "biliary cirrhosis," as did Charcot and Gombault,<sup>10</sup> Harley and Barratt,<sup>34</sup> Richardson,<sup>20</sup> Ogata,<sup>21</sup> Rous and Larimore,<sup>35</sup> MacMahon, Lawrence and Maddock,<sup>36</sup> Moon<sup>37</sup> and others. Similar procedures studied by Litten,<sup>24</sup> Gerhardt,<sup>38</sup> Cameron and Oakley,<sup>39</sup> Bollman and Mann<sup>40</sup> and others were not specifically interpreted as producing "biliary cirrhosis." Quincke,<sup>19</sup> Ogata<sup>21</sup> and Zypkin<sup>41</sup> were reluctant to apply such conclusions from animal experimentation to man, whereas MacMahon and Mallory<sup>23</sup> were inclined to regard the results in man and animals as essentially similar. Of particular interest is the report of Snell, Greene and Rowntree<sup>42</sup> in which they described ascites, extensive collateral circulation and monolobular hepatic fibrosis occurring in 2 dogs after prolonged ligation of the common duct.

Perhaps the most comprehensive recent review of the subject of "biliary cirrhosis" is that of Rossle.<sup>6</sup> He recognized cholestatic "biliary cirrhosis" (due to pure stasis of bile), the more frequent cholangitic "biliary cirrhosis" (due to bile stasis with superimposed infection) and the infrequent cholangiolitic "biliary cirrhosis" (due to toxic alterations of the smallest bile ducts).

29 Kaufmann, E. Lehrbuch der speziellen pathologischen Anatomie für Studierende und Aerzte, ed 6, Berlin, G. Reimer, 1911, pp 593-594.

30 Janowski, W. Beitr z path Anat u z allg Path **11** 344, 1892.

31 Judd, E. S., and Counseller, V. S. J A M A **89** 1751, 1927.

32 Greene, C. H., McVicar, C. S., Snell, A. M., and Rowntree, L. G. Arch Int Med **40** 159, 1927.

33 Weir, J. F., and Snell, A. M. Am J Digest Dis & Nutrition **3** 629, 1936.

34 Harley, V., and Barratt, W. J Path & Bact **7** 203, 1901.

35 Rous, P., and Larimore, L. D. J Exper Med **32** 249, 1920.

36 MacMahon, H. E., Lawrence, J. S., and Maddock, S. J. Am J Path **5** 631, 1929.

37 Moon, V. H. Arch Path **18** 381, 1934.

38 Gerhardt, D. Arch f exper Path u Pharmakol **30** 1, 1892.

39 Cameron, G. R., and Oakley, C. L. J Path & Bact **35** 769, 1932.

40 Bollman, J. L., and Mann, F. C. Ergebn d Physiol **38** 445, 1936.

41 Zypkin, S. M. Virchows Arch f path Anat **262** 791, 1926.

42 Snell, A. M., Greene, C. H., and Rowntree, L. G. Arch Int Med **40** 471, 1927.

Several series of necropsies have demonstrated that so-called biliary cirrhosis following obstruction of the bile ducts is relatively infrequent. Ophuls<sup>43</sup> reported the incidence to be 0.43 per cent, Mallory,<sup>44</sup> 0.29 per cent, and Schumacher,<sup>45</sup> 0.40 per cent.

#### MATERIAL AND METHODS

The cases in this series were chosen from the necropsy material of the section on pathologic anatomy of the Mayo Clinic covering the period from July 1, 1922, to June 30, 1938, inclusive. During this time there were 8,986 necropsies, covering all age groups.

Only those cases were selected in which necropsy showed biliary obstruction and obstructive jaundice. A group of 244 cases (2.7 per cent of the total number in which necropsy records were available) fulfilled these requirements. The records of these cases were reviewed, particular attention being given to (1) the course of the jaundice, (2) any clinical suggestion of antecedent hepatic parenchymal disease, (3) the gross appearance of the liver and biliary tract and (4) any evidence of portal obstruction, such as esophageal varices and ascites. Note was made of the presence of hepatic metastasis, suppurative cholangitis with abscess formation, pyelephlebitis, hepatic vascular lesions, peritonitis, chronic passive congestion and other lesions. These lesions did not serve to exclude cases from the series, since such lesions do not, as a rule, induce hepatic changes which confuse the picture of true hepatic cirrhosis.

The important part of the problem centered about the microscopic studies. It was felt that ultimately the diagnosis of cirrhosis must rest on histologic study. The routine tissue sections of the liver, biliary passages and obstructive biliary lesion, stained with hematoxylin and eosin, were studied. Ordinarily these sections were entirely adequate, but in many instances they were supplemented by other sections from the liver stained by the Van Gieson, Mallory-Heidenhain and Perdrau techniques for connective tissue.

#### OBSERVATIONS AND RESULTS

In this group of 244 cases in which necropsy showed biliary obstruction and obstructive jaundice, almost every type of obstructive biliary lesion was represented. Some form of neoplastic obstruction was present in 64.3 per cent (157 cases). In only 35.7 per cent (87 cases) could the disease be classified as due to a benign type of obstruction. In 48.3 per cent (42 cases) of the latter group the obstruction was due to choledocholithiasis, and in 44.8 per cent (39 cases), to postcholecystectomy stricture.

It was found that the cases could quite easily be grouped into those in which the jaundice, even though fluctuating, was continuously present from its first appearance and those in which the jaundice was intermittently present, i. e., in which there were at least two episodes of

43 Ophuls, W. A Statistical Survey of Three Thousand Autopsies, Stanford University, Calif., Stanford University Press, 1926, pp. 275, 286 and 302.

44 Mallory, F. B. New England J. Med. **206** 1231, 1932.

45 Schumacher, G. A. Am. J. M. Sc. **194** 693, 1937.

jaundice separated by a period in which visible icterus had apparently cleared. Jaundice was continuous in 78.7 per cent (192 cases). In 73.4 per cent (141 cases) of this group the jaundice was associated with neoplastic obstruction whereas in 26.6 per cent (51 cases) it was secondary to benign obstruction. In only 18 per cent (44 cases) was the jaundice intermittent, in 25 per cent of these (11 cases) it was due to neoplastic obstruction, and in 75 per cent (33 cases) it was due to benign obstruction. Eight cases, or 3.3 per cent, could not be definitely included in either group. It is of particular interest that of the 11 cases in which intermittent jaundice was associated with neoplastic obstruction, the obstruction in 7 was due to carcinoma of the ampulla of Vater.

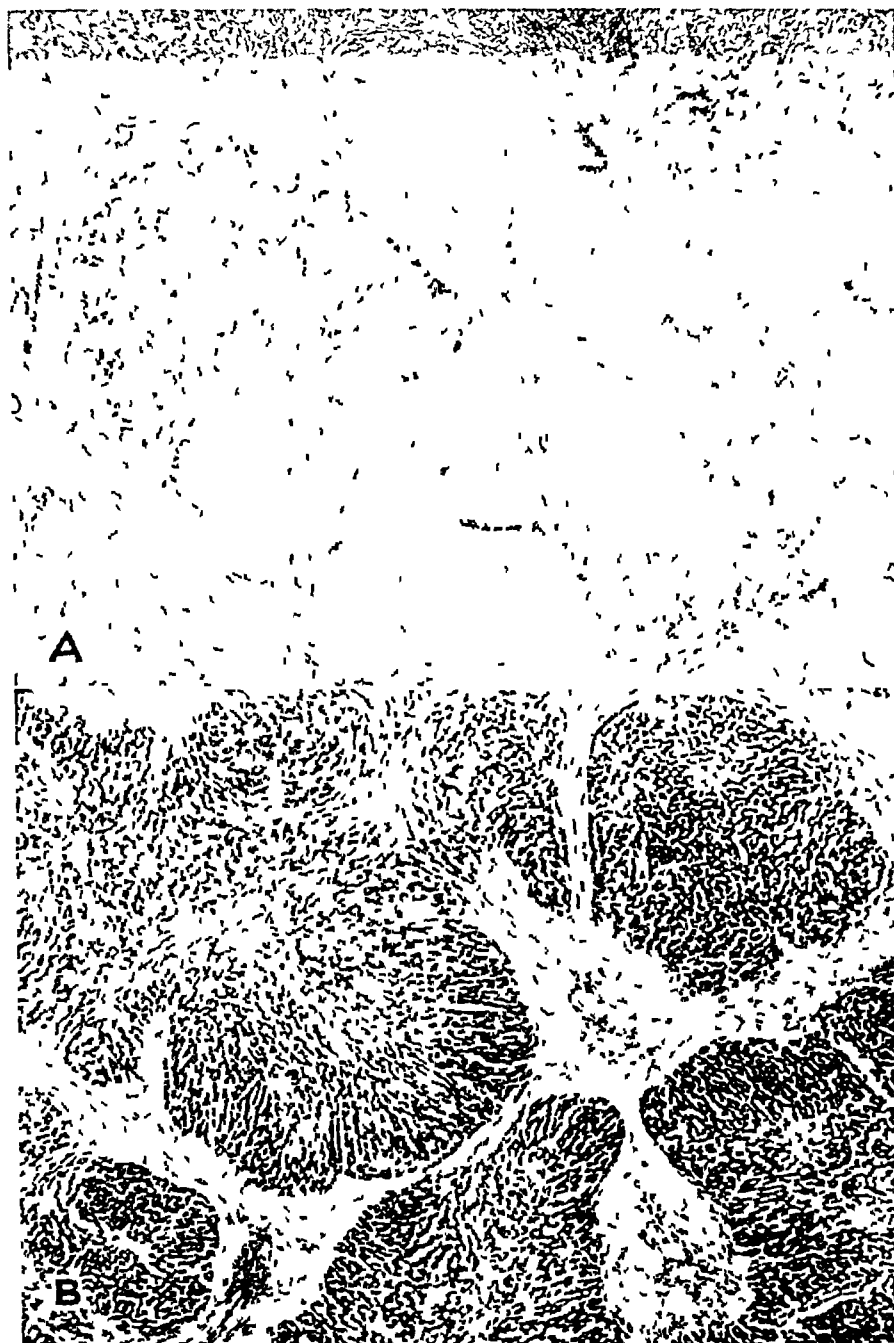
There was a definite difference between these two groups in respect to the duration of life after the first appearance of jaundice. In the group with persistent jaundice 87.9 per cent (124 patients) of those with neoplastic obstruction and 70.6 per cent (36 patients) of those with benign obstruction died within three months after the onset of jaundice. In the group with intermittent jaundice 54.5 per cent (6 patients) of those with a neoplastic obstruction and 54.5 per cent (18 patients) of those with a benign obstruction died more than one year after the first appearance of jaundice.

In this series of 244 cases in which necropsy showed biliary obstruction and obstructive jaundice, true hepatic cirrhosis was present in 8.6 per cent (21 cases). The criteria for this diagnosis have already been set forth as including parenchymal atrophy, fibrosis and nodular parenchymal regeneration.

The obstructing lesion in 10 of these 21 cases was postcholecystectomy stricture, in 6 choledocholithiasis, in 2 carcinoma of the ampulla of Vater, in 1 carcinoma of the gallbladder with invasion of the common duct, in 1 carcinoma of the head of the pancreas and in 1 recurrent carcinoma of the stomach with invasion of the common duct.

There were 7 men and 14 women with true hepatic cirrhosis. There were 4 men in the group with neoplastic obstruction and 3 with choledocholithiasis. The average age of those with postcholecystectomy strictures was 40 years, of those with choledocholithiasis, 57 years, and of those with neoplastic obstruction, 62 years. The average age for the entire group was 50 years.

It is of particular interest that 71.4 per cent (15 patients) of the 21 patients gave a history of intermittent obstructive jaundice. Every patient with benign obstruction gave a history of intermittent jaundice except a patient in the group with postcholecystectomy stricture and another in the group with choledocholithiasis. It is also of interest that the average duration of life after the first appearance of jaundice was 3.8 years for the patients with benign obstruction, 0.5 year for those with neoplastic obstruction and 3 years for the entire group.



*A*, cirrhosis (with portal obstruction, varices with hemorrhage and ascites) from biliary obstruction,  $\times 25$  Recurrent postoperative stricture of the common duct with obstructive jaundice (occurring intermittently for 8 years) *B*, cirrhosis from biliary obstruction,  $\times 25$  Recurrent postoperative stricture of the common duct with obstructive jaundice (occurring intermittently for 49 months)

In the livers of these patients with true hepatic cirrhosis there were the usual gross changes associated with biliary obstruction. A granular or nodular surface was present in 13. Two patients had esophageal varices with serious hemorrhage and ascites. Two patients had well developed collateral venous circulation elsewhere with ascites. In 2 other patients there was ascites without demonstrable varices. One of these also had carcinomatosis of the peritoneum.

Microscopically, the architecture of these livers was altered, sometimes to a marked degree. There were widespread parenchymal degenerative changes, commonly most marked about the central vein but frequently focal and occasionally peripheral. Sometimes actual necrosis was present. It was not unusual to see isolated groups of parenchymal cells in the portal connective tissue. There was a moderate to marked increase in the portal connective tissue, and it was not unusual to see intralobular extension. Particularly noteworthy in these specimens were the evidences of parenchymal regeneration. This had taken place to a degree deserving of the term nodular. In these nodules the sinusoidal pattern and vascular relationships were altered. Bile thrombi were present in every case, and frequently parenchymal and reticuloendothelial cells contained bile pigment. Commonly there was at least an apparent increase in the interlobular bile ducts. Collections of lymphocytes or polymorphonuclear leukocytes were frequently present. Two typical cases are illustrated (figure).

In one of these cases there was a history of a moderate use of alcohol. In another there was concurrent mild exophthalmic goiter. Neither of these factors was regarded as sufficient to influence the hepatic changes found. Syphilis was ruled out by the Kolmer or the Kline and the Kahn test in all but a single case. In the latter case there was nothing in the clinical or necropsy records to suggest syphilis. In an additional case there was a six year history of dyspepsia, colic and vague spells of pruritus. Finally there was jaundice for two months, and at necropsy a carcinoma of the common duct was encountered. This case was discarded because of the suggestion of antecedent portal cirrhosis. The past histories of the other cases were entirely negative in this respect.

#### COMMENT

In a review of the extensive literature it appears that so-called biliary cirrhosis is recognized by its proponents in livers which have endured biliary stasis and which have parenchymal degenerative changes of various sorts, an increase in the portal connective tissue, an apparent increase in the interlobular ducts, bile thrombi and collections of cells, such as lymphocytes and polymorphonuclear leukocytes, in the portal connective tissue. Emphasis is clearly laid on bile stasis, parenchymal degeneration and fibrosis of some degree.

That these changes are commonly associated with biliary obstruction in man and in experimental animals is not doubted. In our study of 244 cases in which necropsy showed biliary obstruction and obstructive jaundice it was found that most of the changes listed in the foregoing paragraph were almost always present.

The outstanding feature in the liver which has endured biliary obstruction is parenchymal degeneration. Quincke<sup>19</sup> emphasized this pertinent point. Similarly, the results of parenchymal degeneration frequently dominate the clinical picture. It is for this reason that experience dictates a guarded prognosis for these patients unless the biliary obstruction can be relieved and hepatic recovery permitted. Quincke<sup>19</sup> and Rolleston<sup>27</sup> were inclined to regard the interstitial proliferation as of only histologic interest and without influence on the clinical course of the disease. Eppinger<sup>46</sup> interpreted the late picture of uncomplicated obstructive jaundice as primarily one of parenchymal atrophy with condensation of connective tissue. Certainly a diagnosis of biliary cirrhosis according to the current concept of the term adds little to the interpretation of either the clinical or the pathologic picture. It seems that conditions of this type might be described more suitably as hepatic atrophy. This term would place the emphasis where it may rightfully belong—on the parenchymal degeneration which is associated with biliary obstruction and jaundice. Further, it is appreciated clinically that at least certain phases of hepatic function are impaired in the presence of obstructive jaundice, even though there are recognized limitations to the clinical tests of hepatic function. The term “hepatic atrophy” would be compatible with an impairment of hepatic function and might even imply it. It should not be confusing to speak of hepatic atrophy in the presence of a normal or greater than normal weight of the liver since such an organ enduring obstructive jaundice can still be atrophic to microscopic examination and be impaired in its physiologic functions. The latter factors seem more important fundamentally than the gross weight of the liver, which is notoriously unpredictable and frequently difficult to evaluate in considering the course of the disease in retrospect.

In this study 21 cases of a condition consisting in biliary obstruction, obstructive jaundice and true hepatic cirrhosis were found. The criteria for the latter diagnosis have been stated. It is clear that there is a definite distinction between these cases and cases of what is called “biliary cirrhosis” in the literature. The distinctive feature is the nodular parenchymal regeneration which is present in addition to the parenchymal atrophy and fibrosis. Just as progressive hepatic parenchymal degeneration may lead to death, so may sufficient parenchymal regeneration conceivably prevent an untimely death.

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46 Eppinger, H. Beitr. z. path. Anat. u. z. allg. Path. **31** 230, 1902, footnote 7.

It is of particular interest concerning the 21 cases of biliary obstruction, obstructive jaundice and true hepatic cirrhosis that there was a history of intermittent jaundice in 71.4 per cent (15 cases). The average duration for the entire group from the first appearance of jaundice to death was 3 years. The combination of these facts suggests intermittent episodes of parenchymal destruction over a relatively long period with intervals of relief from jaundice, permitting an opportunity for parenchymal repair. Theoretically, at least, intermittent destruction and intermittent repair present a favorable situation for the production of true hepatic cirrhosis.

In the remaining cases, 28.6 per cent (6 cases), there was continuous jaundice. The jaundice was fluctuating in 4 of these cases and only mild to moderate in 2. The average duration from the first appearance of jaundice to death in these cases was a little over 0.6 year. It is apparent that parenchymal regeneration may occasionally occur in the presence of continuous obstructive jaundice.

It has occasionally been stated in the literature that biliary obstruction, obstructive jaundice and hepatic cirrhosis of the Laennec type may infrequently occur together. Litten,<sup>24</sup> Janowski,<sup>30</sup> Quincke,<sup>19</sup> Rolleston,<sup>27</sup> Karsner<sup>47</sup> and others have expressed such an opinion. Ford<sup>18</sup> even regarded "hepatic contraction" with ascites and venous collateral circulation as relatively frequent in "biliary cirrhosis." The objection might well be raised that the entire group of 21 cases presents two concurrent but unrelated lesions, namely, hepatic cirrhosis and biliary obstruction. One case has been mentioned in a previous section of this paper which was ruled out because this seemed possible. Recently Bloomfield<sup>48</sup> graphically reemphasized the fact that hepatic cirrhosis of the Laennec type is commonly latent and asymptomatic "until the final crash of hepatic insufficiency." Hence the possibility that the hepatic cirrhosis was present before the onset of biliary obstruction cannot be denied. It would be of great interest to know the state of the liver at the time of the initial operation in the patients in whom postcholecystectomy stricture and true hepatic cirrhosis subsequently were found. Unfortunately, these patients without exception originally were operated on elsewhere.

It is of indirect value to refer to the incidence of hepatic cirrhosis of all types in persons examined post mortem. Ophuls<sup>43</sup> reported an incidence of 5.5 per cent, Mallory,<sup>44</sup> 5.9 per cent, and Schumacher,<sup>45</sup> 3.7 per cent. Rossle<sup>6</sup> cited a number of other series, including his own, in which the highest incidence of cirrhosis was 4 per cent.

47 Karsner, H. T. *Human Pathology*, Philadelphia, J. B. Lippincott Company, 1926, p. 682.

48 Bloomfield, A. L. *Am J M Sc* 195:429, 1938.



It is of interest that the incidence of hepatic cirrhosis in this series of cases of biliary obstruction and obstructive jaundice is 86 per cent. This is perhaps significantly greater than the incidence of hepatic cirrhosis of all types in the usual necropsy series.

These data suggest that the relationship between biliary obstruction, obstructive jaundice and true hepatic cirrhosis may be more than coincidental. It is suggested that in such cases the condition be called "cirrhosis from biliary obstruction" and that the phrase "biliary cirrhosis" be dropped as confusing and misleading.

#### SUMMARY

It is suggested that hepatic cirrhosis be defined as including parenchymal degeneration, fibrosis and nodular parenchymal regeneration.

From a series of 244 cases of biliary obstruction and obstructive jaundice, 21 cases, or 86 per cent, were separated out in which these conditions were associated with hepatic cirrhosis as defined in the foregoing paragraph. In 10 of these 21 cases the biliary obstruction was due to postoperative stricture of the common duct, in 6 cases to choledocholithiasis, in 2 cases to carcinoma of the ampulla of Vater and in 3 cases to other malignant lesions.

An intermittent type of obstructive jaundice was present in 15 of these 21 cases, or 71.4 per cent. The average case duration from the first onset of jaundice to death was 3 years. These factors may be involved in the production of regeneration in these cases.

It is suggested that the term "biliary cirrhosis" be dropped and that for the infrequent combination of biliary obstruction, obstructive jaundice and true hepatic cirrhosis the designation "cirrhosis from biliary obstruction" be employed.

Cases in which hepatic parenchymal damage without signs of regeneration follows obstruction of the bile ducts should be classified as instances of hepatic atrophy.

# EFFECT OF THOROTRAST (COLLOIDAL THORIUM DIOXIDE) ON EPENDYMAL LINING AND RELATED PARTS OF THE BRAIN

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Thorotrast,<sup>1</sup> a colloidal preparation of thorium dioxide, is recommended by some as a contrast medium for ventriculographic work preferable to air. Radovici and Meller<sup>2</sup> were the first to use thorotrast for the visualization of the ventricular cavities. They described their observations in a series of papers in which they expressed the opinion that the elimination of this substance from the cerebrospinal pathways was carried out by way of the blood stream and lymphatics. In their later reports they concluded that this elimination at best must be very slow, and by roentgen and histologic studies they demonstrated the persistence of thorotrast in the ventricular system of monkeys a year after injection. In their investigations they described the accumulation of this substance in the subarachnoid spaces and its adherence to the ependymal surface, and they emphasized the absence of penetration into the parenchyma of the brain and the absence of perivascular reaction. In a more recent contribution<sup>2c</sup> they described thorotrast as a completely inert substance, but on the basis of its slow elimination and the changes it obviously produced in the brain they advised extreme caution in its use as a diagnostic medium. Jacobi, Lohr and Wustmann,<sup>3</sup> however, considered the use of thorotrast in its present form to be a safe procedure. Freeman and his co-workers<sup>4</sup> also favored the use of thorotrast for the purpose under consideration and reported that one of their patients showed no clinical evidence of harmful effects twenty months after the

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From the Laboratories of the Mount Sinai Hospital

1 Thorotrast, preliminary report of the Council on Pharmacy and Chemistry, J A M A **99** 2183, 1932

2 (a) Radovici, A, and Meller, O. Bull Acad de med, Paris **107** 314, 1932, (b) Presse med **40** 1933, 1932, (c) Rev neurol **1** 541, 1933 (d) Radovici, A, Bazgan, I, and Meller, O. Compt rend Soc de biol **114** 207, 1933 (e) Radovici, A, and Meller, O. Presse med **42** 153, 1934

3 (a) Wustmann, O. Deutsche Ztschr f Chir **238** 530, 1933 (b) Jacobi, W, Lohr, W, and Wustmann, O. Ueber die Darstellung des zentralen und peripheren Nervensystems im Rontgenbild, Leipzig, Johann Ambrosius Barth, 1934

4 Freeman, W, Schoenfeld, H H, and Moore, C. J A M A **106** 96, 1936

injection In a more recent study Freeman<sup>5</sup> investigated human brains coming to autopsy at different periods after the injection of thorotrast He found that the earliest changes, which became evident within an hour after the introduction of thorotrast, were swelling of the epithelium of the choroid plexus and adherence of granules of thorotrast to its surface In a brain which came to autopsy twenty-four hours after the injection of thorotrast a leukocytic infiltration with some destruction of the epithelium of the choroid plexus was noted The ependyma, at this time, showed beginning exfoliation and an aggregation of macrophages and leukocytes over the free surface of the ependymal lining The later development of the process is not clear from the description given by Freeman He stressed the fact that in the presence of obstructive hydrocephalus there is a severe, persistent inflammatory process with destruction of the ependyma He assumed that in the absence of obstruction thorotrast disappears from the ventricular system within four days, but he did not present clearcut evidence to support this view He added further that in patients with obstruction the choroid plexus was restored to a normal condition within four days after the injection

Radović, Bazgan and Meller<sup>6</sup> and Jacobi, Lohr and Wustmann<sup>3b</sup> reported changes caused by thorotrast injected into the ventricles and emphasized especially a macrophagic reaction, destruction of the ependymal lining and the freedom of the brain tissue from parenchymatous alterations

Reeves and Stuck,<sup>7</sup> on the other hand, in a general review of the subject concluded that the dangers involved in the use of this substance outweighed the possible advantages They carried out thorough roentgen and histologic studies on the behavior of thorotrast in monkeys and reached the conclusion that "no consequential transportation of the substance from the subarachnoid spaces into the blood stream occurs" They discussed the radioactivity of thorotrast, which they felt was an important contraindication to its use and a likely factor in the injurious effect In a later paper<sup>8</sup> they described the development of hydrocephalus after injection of thorotrast in cats, dogs and monkeys

Alexander, Jung and Lyman,<sup>9</sup> working with dogs, found damaged ependyma and alterations which they regarded as evidence of the ependymal origin of the macrophages involved in the reaction to thorotrast

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5 Freeman, W Arch Neurol & Psychiat **38** 340, 1937

6 Radović, A , Bazgan, I , and Meller, O Encephale **28** 726, 1933

7 Reeves, D L , and Stuck, R M Medicine **17** 37, 1938

8 Stuck, R M , and Reeves, D L Arch Neurol & Psychiat **40** 86, 1938

9 Alexander, L T , Jung, S , and Lyman, R S Arch Neurol & Psychiat **32** 1143, 1934

It is obvious from the foregoing citations that the question as to whether the use of thorotrast in human beings is entirely without hazards is not fully answered, and therefore it is thought advisable to record all reliable observations bearing on this problem. The case which will now be reported is of value because of a detailed histologic study made on the brain a month after the introduction of thorotrast by ventricular puncture.

#### REPORT OF A CASE

A 5 year old girl was brought to the hospital Feb 24, 1936. Two weeks after an apparently normal birth, stiffness of her left arm was observed. The stiffness did not persist but occasionally recurred in attacks of convulsions with cyanosis. At six weeks she experienced several attacks of generalized convulsions with cyanosis. By the age of 3 months the seizures had stopped. Development then seemed to proceed normally—the child sat up at 6 months, stood at 10 months, walked at 14 months and talked at 18 months. When she began to walk, it was noted that she dragged her left leg. At the age of 2 years, she was struck on the head by a dropping toilet seat. She showed no marks of local injury, but ten minutes later, while being fed, she suddenly vomited, became unconscious and was seized by a severe generalized convulsion. She was taken to a hospital where a general anesthetic was required to control the convulsion. She regained consciousness several hours later. A roentgen examination of the skull revealed no fracture. Following this episode, the child became subject to convulsive seizures with or without preceding nausea or vomiting. The attacks recurred at intervals of several months and were usually associated with infections of the upper respiratory tract. At the age of 3 years (in April 1933), during the course of an acute illness with fever and a rash (the exact diagnosis could not be ascertained), she passed through a severe seizure, with her temperature rising to 108 F. At the age of 4½ years, during another severe seizure, with her temperature rising to 105 F, a spinal tap yielded bloody fluid. She remained unconscious for several days, the left leg being held in flexion. She then continued in a semi-comatous state for ten days. As she was regaining consciousness, it was noted that she spoke in a parrot-like fashion and was unable to form complete sentences. She had become easily frightened and was difficult to manage.

The child was well nourished. She did not respond to simple commands and did not display any emotional reaction. The left palpebral fissure was wider, and the left pupil larger, than that on the right. The fundi were normal. There were paresis of the central type of the left side of the face and spastic paralysis of the left arm and leg. All deep reflexes were increased, and the abdominal reflexes were diminished on the left side. There was a Babinski sign on the left.

The cerebrospinal fluid at the time of admission was normal. The Wassermann tests of the blood and spinal fluid were negative.

On admission an injury sustained at birth and a developmental defect of the right prefrontal area were considered as diagnoses, with some vascular anomaly or a porencephalic defect also being suggested as possibilities. Four days after admission encephalographic examination revealed marked internal hydrocephalus, the right ventricle being larger than the left. The third ventricle was distended. This examination was not altogether satisfactory and March 15 ventriculographic examination was again made and showed marked internal hydrocephalus. Following this procedure the child experienced numerous convulsive seizures, and slight

papilledema appeared in the right disk. As the papilledema advanced and became bilateral a midline tumor was accepted as a likely diagnosis, particularly since, on March 24, ptosis of the left lid and paresis of the upper and outward movement of the left eye developed.

On March 27 a ventriculographic examination was made with the use of thorotrast. It disclosed huge lateral ventricles but no trace of the third ventricle. The next day, March 28, four weeks after admission, a suboccipital craniotomy was performed. When the cisterna magna was opened, the foramen of Magendie



Fig 1—The gross appearance of the brain, showing hydrocephalus with atrophy of the right hemisphere

was found to measure 0.5 cm. in diameter. The fourth ventricle was also enlarged. A fragment of pia-arachnoid from the cisterna magna was removed for histologic study. This showed chronic reactive leptomeningitis secondary to "subarachnoid hemorrhage." Following the operation the patient's condition became progressively worse. The spinal fluid, which had been clear on repeated examinations, became cloudy twenty-five days after operation. The temperature rose to 105.2 F. Violent convulsive seizures set in, during which the child assumed a posture of decerebrate rigidity. She died during one of these seizures, on April 23, eight weeks after admission and four weeks after operation following ventriculographic examination by means of thorotrast.

*Postmortem Examination of Brain*—The right cerebral hemisphere was smaller in all respects than the left. On sectioning, the enlargement of the ventricles was brought to view the smaller right hemisphere having the larger ventricle (fig 1). The foramina of Monro were markedly dilated, the aqueduct of Sylvius and the fourth ventricle were also dilated but to a lesser degree. The cerebellum appeared normal.

*Microscopic Examination of Brain*—The architecture of the cerebral cortex was markedly disturbed. This was due to a reduction in the number of nerve cells and subcortical fibers and to a relative increase in glial elements. The gliosis



Fig 2—Section of the pons showing almost complete disappearance of the fibers of the pyramidal tract on the right side

affected all glial elements. In some sections the gliosis appeared most marked in specific layers of the cortex. The deeper layers of the cortex were disorganized, owing to extensive loss of cellular elements. These changes were present in both hemispheres but most marked in the right.

Scarlet red stains showed small deposits of fat scattered throughout the cortex and subcortex but concentrated chiefly about blood vessels. The subependymal zone was free from fat.

Sections of the midbrain, pons and medulla oblongata revealed marked reduction in the volume of the fibers of the pyramidal tract on the right side (fig 2). In addition, stellate foci of demyelination were found in the pes pedunculi on each side.

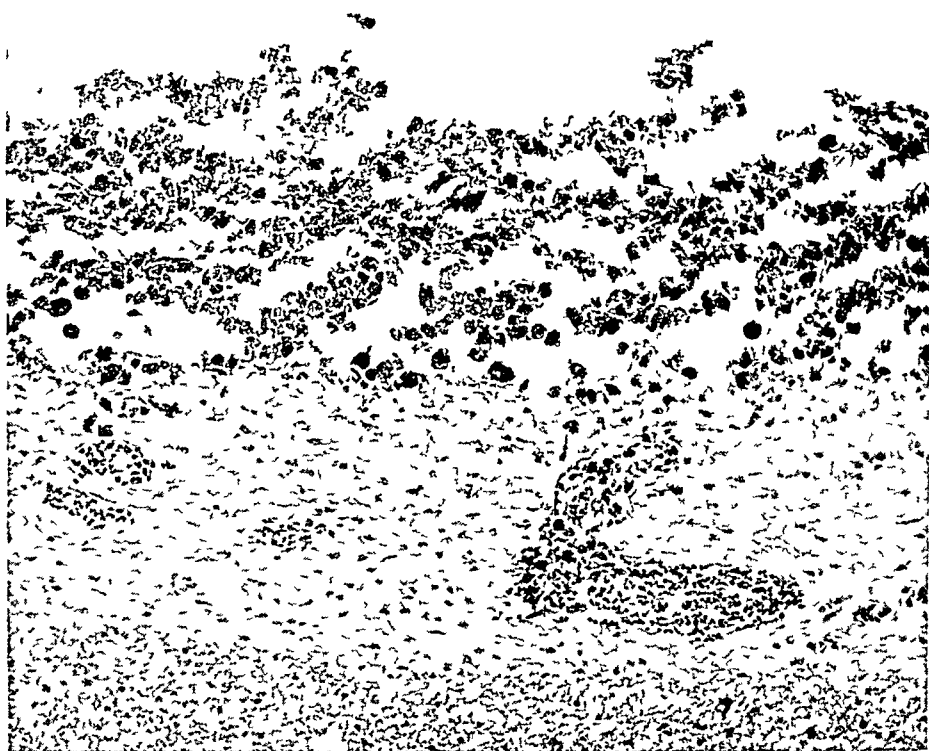


Fig 3—Section displaying the ventricular lining of the posterior horn of the right lateral ventricle. It is almost completely desquamated, and the ependymal cells are rounded up to form macrophages, which contain thorotrast



Fig 4—Section of the ventricular lining showing ependymal granulations and perivascular infiltration in the subependymal zone



Fig 5—Macrophages, containing granules of thorotrast, enveloping a large vessel in the subependyma

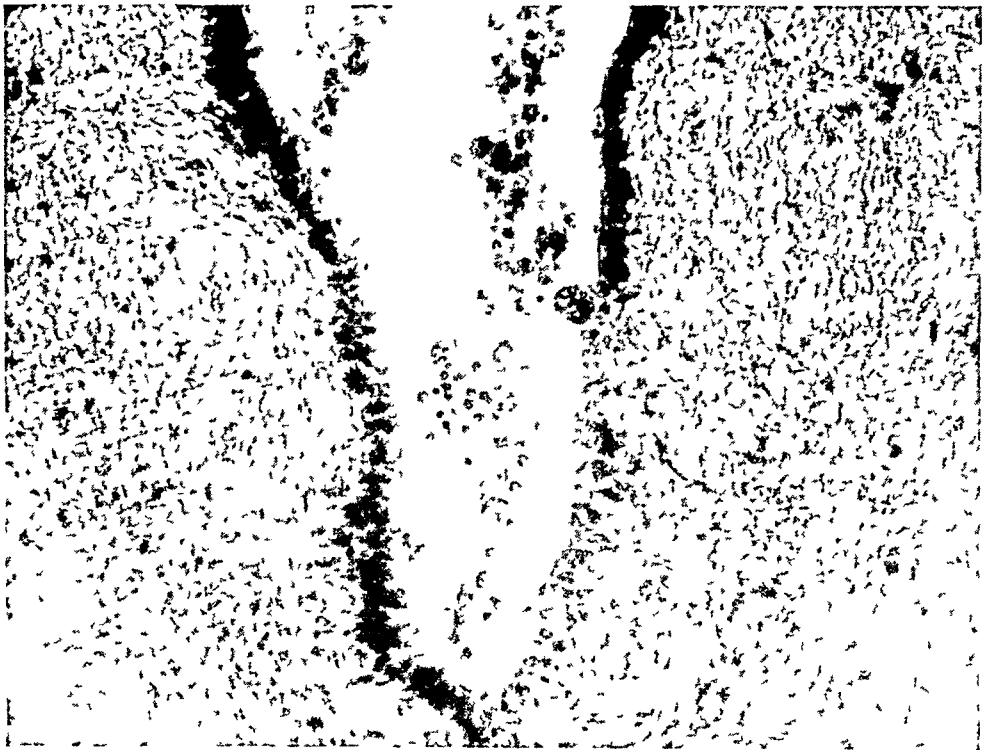


Fig 6—Section of the midbrain showing part of the aqueduct of Sylvius with intact ependymal lining and thorotrast-containing macrophages free in the aqueduct



The ependymal cells were swollen and vacuolated, some containing granules of thorotrast. The underlying supporting tissue was densely infiltrated by macrophages containing particles of thorotrast. At one site there was a large accumulation of macrophages about blood vessels. Covering the ependyma were large aggregates of thorotrast-laden macrophages. The ependymal lining for the most part, however, remained intact, presenting a sharp contrast to the surrounding masses of macrophages on the surface and in the subependymal connective tissue.

In the right lateral ventricle the ependymal lining was almost entirely desquamated (fig 3). Lying free in the ventricular cavity were large numbers of macrophages containing granules of thorotrast. Ependymal granulations occurred



Fig 7—Preparation showing ependymal cells in the process of desquamation

at frequent intervals (fig 4). The subependymal blood vessels showed a striking perivascular infiltrate of thorotrast-laden macrophages and lymphocytes. This reaction was usually found near an area of severely damaged ependyma (figs 3, 4 and 5).

The ependymal lining in the left lateral ventricle and in the third ventricle showed milder forms of the same pathologic process. In the aqueduct of Sylvius the ependymal lining was intact except for scattered foci of injury. There were, however, accumulations of macrophages resting on the intact ependyma (fig 6).

At several points there appeared thorotrast-containing cells suggestive of desquamating ependyma cells (fig 7).

The pia-arachnoid showed varying changes at different sites. The interpeduncular space was crowded with a large quantity of thorotrast-laden macrophages. Scattered macrophages were found in the subarachnoid spaces over the convexity of the brain and surrounding the spinal cord.

Macrophages did not appear in the pachionian bodies or in the dural sinuses.

#### SUMMARY

In the case herein described the patient lived for twenty-seven days after the introduction of thorotrast into the ventricular system. A large amount of this substance was still present in the ventricular cavities and in the subarachnoid spaces, though there was no obstruction to the flow of cerebrospinal fluid, as demonstrated by the fact that a considerable accumulation of thorotrast-laden macrophages was observed in the subarachnoid spaces.

The observation of particles of thorotrast in macrophages is at variance with the assumption made by Freeman that in the absence of obstruction the substance disappears from the ventricular cavities within four days. It is, however, in accord with the evidence presented by Radović and Meller,<sup>2e</sup> Stuck and Reeves<sup>s</sup> and Jacobi and his co-workers.<sup>3b</sup>

The thorotrast caused extensive damage in the ependymal lining, especially in the right lateral ventricle, into which the substance had been directly introduced. The pathologic changes consisted of desquamations of ependymal lining and extensive macrophagic accumulations in the ventricular cavities, about the choroid plexus and in the subarachnoid spaces. Thorotrast-laden macrophages were also found in the perivascular spaces of the subependymal zone. In several areas ependymal cells filled with particles of thorotrast could be seen becoming detached and rounding up (fig. 7). This points to the ependymal cells as the probable source of origin of the macrophages, an observation which is in accord with the views of Alexander, Jung and Lyman.<sup>9</sup>

#### CONCLUSIONS

Thorotrast (colloidal thorium dioxide) is not readily excreted by the central nervous system and may remain in the ventricular cavity for long periods (in this case, for twenty-seven days) in spite of the absence of obstruction to the flow of the cerebrospinal fluid.

Thorotrast produces extensive inflammatory and destructive changes in the ependymal lining. The source of the macrophages which ingest the granules of thorotrast may be traced in part at least to ependymal cells.

The use of thorotrast in patients for the visualization of ventricular cavities is unsafe.

# Case Reports

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## HODGKIN'S DISEASE WITH INVASION OF PERICARDIUM AND GALLBLADDER

Review of the Literature and Report of a Case with Autopsy

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Since the original description by Hodgkin of the disease which now bears his name, a vast literature has accumulated. Although the etiologic factors are still unknown, the definite criteria which have been established for making the diagnosis would probably exclude many of the cases earlier reported to be instances of this disease. Although the lesions are found primarily in lymphoid tissue, various writers have described lesions of Hodgkin's disease in practically every organ in the body. Since lesions of the pericardium and of the biliary tract are among the rarest, it was thought that the case to be reported now warranted recording, and that a review of the literature on these lesions would be interesting.

### LITERATURE

Reed<sup>1</sup> reported a case of Hodgkin's disease in which several nodular lesions with the typical microscopic appearance were found in the pleuro-pericardial membrane. Yamasaki<sup>2</sup> reported a "sarcoma of the thymus" breaking through the parietal pericardium at numerous places. Microscopically the tumor had polymorphous characteristics consistent with the lesions of Hodgkin's disease. Karsner<sup>3</sup> agreed with Yamasaki in the belief that Hodgkin's tissue can be transformed into malignant polymorphous cell sarcoma, and he so classifies the condition observed by Yamasaki. Meyer<sup>4</sup> reported a growth invading the pericardium which grossly resembled lymphosarcoma. Though no mention is made of pericardial sections, the adjacent lymph nodes microscopically showed "malignant granuloma", the cellular description is consistent with that in the preceding case. Yates and Bunting<sup>5</sup> mentioned an acute condition macroscopically resembling a malignant tumor with many nodular lesions in the visceral pericardium and "typical Hodgkin's granulomatous infiltration microscopically". Other cases reported in the older literature and cited in extensive reviews of Hodgkin's disease are questionable either because of the lack of microscopic descriptions or because the histologic descriptions indicate that they are probably not instances of Hodgkin's disease.

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From the Department of Pathology, Duke University School of Medicine

1 Reed, D. Johns Hopkins Hosp. Rep. **10** 133, 1902

2 Yamasaki, M. Ztschr. f. Heilk. **25** 269, 1904

3 Karsner, H. T. Arch. Int. Med. **6** 175, 1910

4 Meyer, O. Frankfurt Ztschr. f. Path. **8** 343, 1911

5 Yates, J. L., and Bunting, C. H. J. A. M. A. **64** 1953, 1915

More recently Terplan and Mittlebach<sup>6</sup> described lymphogranulomatous infiltration of the pericardium with "concretio cordis cum pericardio" in their case 2. In their case 10 they found granulomatous nodules in the epicardium over the right and left ventricles, and a mass, 6 by 4 by 2 cm, in the epicardium encircling the origin of the great vessels. Their microscopic descriptions do not include pericardial sections but only typical lesions in cervical lymph glands. The lesion in case 2 is illustrated.

Rimbaud<sup>7</sup> described a case in which there was extensive invasion of the subepicardial tissues and of the parietal pericardium. Dalous, Fabre and Pons<sup>8</sup> reported a gelatinous infiltration over the ventricles at the exit of the great vessels with involvement of the parietal pericardium. The descriptions of the latter 2 cases and the photomicrographs of the lesions leave no doubt of the diagnosis of Hodgkin's disease. Of these 8 cases, the last 3 alone present a degree of involvement comparable to that in the present case.

Meyer<sup>4</sup> reported invasion of the common bile duct by a "tumor" microscopically consistent with Hodgkin's tissue, the gallbladder was not involved. Stahr and Synwoldt<sup>9</sup> reported Hodgkin's tissue in the common and cystic ducts with compression of the lumens, two lymph nodes at the neck of the gallbladder showed typical microscopic lesions. The gallbladder was soft, filled with bile and apparently normal. No other instances of involvement of the biliary tract apart from compression of the ducts by enlarged lymph nodes were found.

#### REPORT OF CASE

A 35 year old white man, a farmer and married, was admitted to Duke Hospital, June 17, 1935, complaining of pain in the left side of the chest and swelling in the neck, present for six weeks.

The family, marital and past histories of the patient were noncontributory.

About six weeks before admission, a sharp pain suddenly developed in the left shoulder and spread along the clavicle to the anterior part of the neck and sternum. After several days a painful red swelling appeared in the left cervical region. The patient was totally incapacitated from the outset, with high sustained fever, weakness, night sweats and an indefinite loss of weight. Tachycardia and edema of the hands, face and feet developed.

On admission the patient was acutely ill, with a temperature of 39 C (102.2 F), a pulse rate of 120, a respiratory rate of 22 and blood pressure of 112 systolic and 55 diastolic. The right pupil was larger than the left, the tonsils were moderately enlarged and infected, and the left was larger than the right.

A smooth red edematous swelling of the lower two thirds of the left antero-lateral aspect of the neck extended down to involve the wall of the chest and obliterated the paraclavicular and episternal fossae. It was indurated and not tender.

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6 Terplan, K, and Mittlebach, M. *Virchows Arch f path Anat* **271** 759, 1929.

7 Rimbaud, P. *Ann d'anat path* **11** 43, 1934.

8 Dalous, Fabre, J, and Pons, H. *Arch d mal du cœur* **29** 89, 1936.

9 Stahr, H, and Synwoldt, I. *Med Klin* **1** 404, 1922.

The axillary lymph nodes were enlarged, firm and discrete, with the largest, on the left side, measuring 4 by 6 cm. A tender left submaxillary node was the only other palpable node. The left upper anterior thoracic wall was prominent, and signs of consolidation were noted. The heart did not appear to be enlarged. The sounds were faint, and there was a pericardial friction rub. The other physical findings were negative. No jaundice was present.

The hemoglobin content was 12.3 Gm (Sahli), the red blood cell count, 3,650,000, the white blood cell count, 11,800, with 81 per cent polymorphonuclear neutrophils, 1 per cent eosinophils, 9 per cent small lymphocytes, 16 per cent large lymphocytes and 3 per cent monocytes. The results of examinations of the urine, stool and sputum, the Wassermann and Kahn reactions and the blood culture were negative. An electrocardiogram showed only sinus tachycardia. Fluoroscopic and roentgen examination of the chest showed diffuse infiltration of the upper lobe of the left lung and a very small amount of fluid at the costophrenic angle. The heart was large and rounded, and the pulsations were feeble. A diagnosis of Hodgkin's disease was made from sections of a lymph node removed from the left axilla.

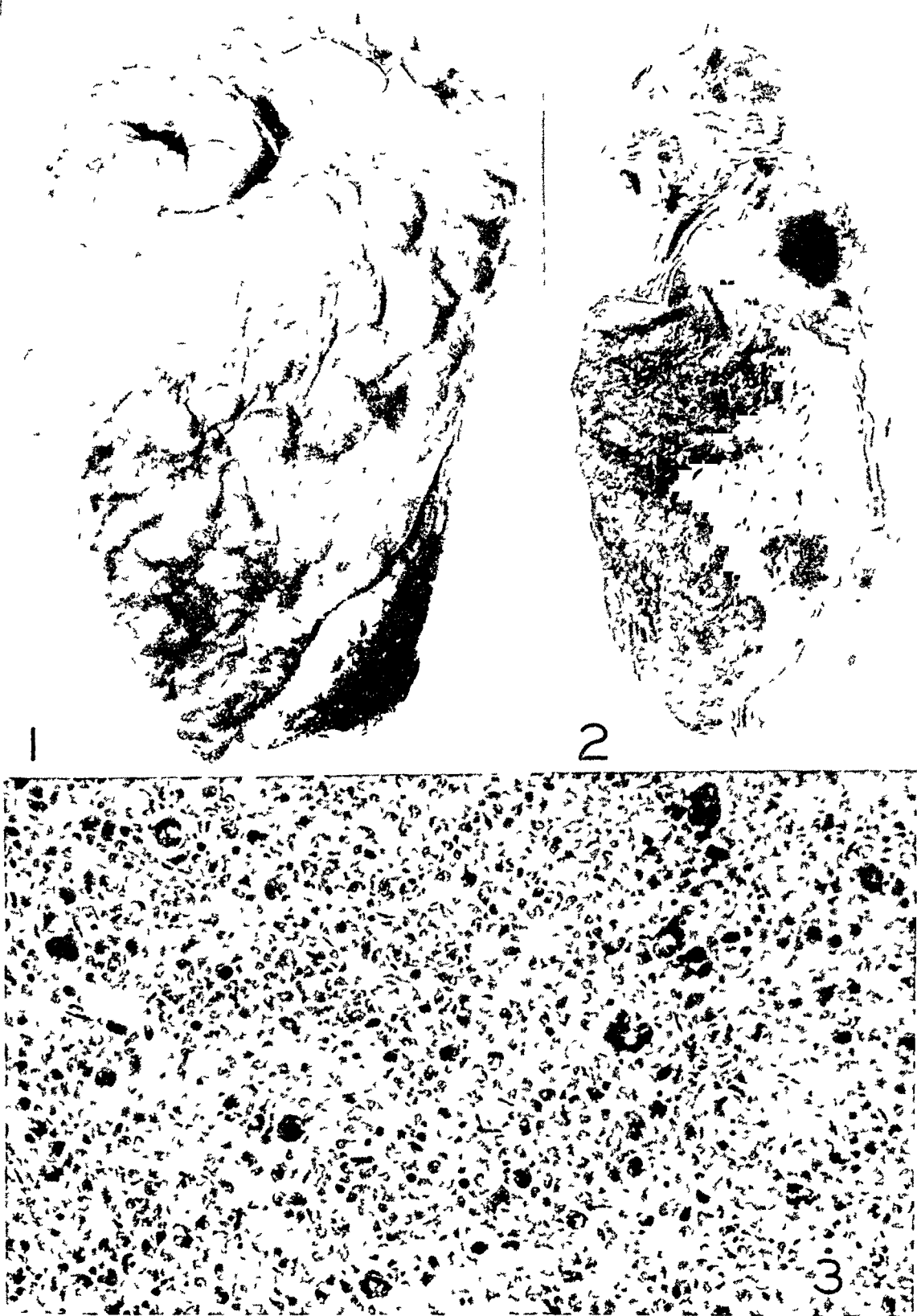
The fever continued, with the temperature ranging between 39 and 40 C (102.2 and 104 F), showing small daily fluctuations, and there was a correspondingly high pulse rate. The white blood cell count ranged from 12,000 to 21,000, averaging about 90 per cent polymorphonuclear neutrophils. The hemoglobin content rapidly fell to 6.5 Gm. The patient began to produce some bloody sputum, which was negative for acid-fast and other organisms. He received roentgen therapy, consisting of 130 to 200 roentgens applied over the left upper part of the chest and neck, four times at two day intervals, with little change in the roentgen or physical findings. He died in coma approximately nine weeks after the onset.

*Postmortem Observations*—The anatomic diagnosis was fulminating Hodgkin's disease, with invasion of mediastinal, cervical, axillary and retroperitoneal lymph nodes and of the left sternocleidomastoid muscle, pericardium, epicardium, myocardium, spleen, left lung, diaphragm, thyroid, gallbladder, mediastinum and trachea, focal necrosis in the spleen, left lung and lymph nodes, fibrinous pericarditis and pleurisy, with fibrous pleural adhesions, adenoma of the thyroid, fibrosis of the testis.

An autopsy was made five hours after death. When the thorax was opened, there was encountered a pale nodular mediastinal mass, firm in consistency and 8 cm in diameter. At the point where the aorta issued from the pericardium there was a layer of soft, almost fluctuant, white material, 2.5 cm thick. The pericardium was 8 mm thick and contained 125 cc of thin bloody fluid, it presented the picture of fibrinous pericarditis. The entire serous surface was covered with shaggy pale translucent nodules, firm to the touch, the largest being 2 cm in diameter.

The heart weighed 300 Gm. A layer of bright yellow fat with a maximum thickness of 4 mm lay between the greatly thickened epicardium and the myocardium. It was more prominent over the right ventricle (fig 1).

The pleural surfaces were bound together with fine, easily broken fibrous adhesions except over the left apex, where the surfaces were densely adherent. There was no free pleural fluid. The left lung weighed 730 Gm, the upper lobe was firm, rubbery and uniformly pale gray. The lymph nodes at the hilus were large and soft and contained many gray granular areas. The right lung was normal and weighed 500 Gm. The entire mucosa of the trachea, beginning very sharply 6 cm below the vocal cords, was infiltrated with a soft reddish growth, granular and friable, which partially obstructed the lumen of the right major bronchus.



1, heart, anterior surface 2, gallbladder in cross section 3, tissue from cervical region involved in Hodgkin's disease, Dominici stain,  $\times 200$

About 100 cc of necrotic material resembling thin pus came from the left side of the neck, smears revealed no polymorphonuclear leukocytes or organisms. A great mass of soft, friable, necrotic lymphoid tissue in the cervical region somewhat resembled caseous tuberculous tissue. The sternocleidomastoid muscle was hard and was infiltrated with dense white tissue.

The liver extended 4 cm below the costal margin and weighed 1,760 Gm. The spleen was enlarged, weighing 220 Gm, and had soft, bulging red pulp. At the junction of the cystic duct with the gallbladder was a 2 cm mass of firm white tissue, covered with mucosa on one side and peritoneum on the other, the duct was not obstructed (fig 2). The mesentery contained numerous rubbery nodes up to 1.5 cm in diameter.

The entire abdominal aorta and the common iliac arteries were surrounded by a mass of firm nodes, averaging 2 cm in length, which did not constrict the lumens. The other organs showed nothing of importance. The brain and spinal cord were not examined because permission to do so was refused.

Cultures of the spleen and of the purulent material from the neck revealed no pathogenic organisms. (Since this paper was prepared, cultures of *Brucella* have been recovered in this laboratory from material from 4 patients clinically and pathologically presenting the picture of Hodgkin's disease.<sup>10</sup> In the present case cultures were not made specifically for that organism.)

Tissues were fixed in Zenker's fluid containing solution of formaldehyde U S P instead of acetic acid (Helly's modification) and in solution of formaldehyde U S P, diluted 1:10. Sections were stained with hematoxylin-eosin, Van Gieson, Masson, Mallory, Heidenhain, Pappenheim, Dominici,<sup>11</sup> Giemsa, Kingoun acid-fast<sup>12</sup> and MacCallum bacterial<sup>13</sup> stains.

The axillary lymph node removed twelve days before death and preceding roentgen therapy revealed fibrosis, hyperplasia and focal necrosis. Eosinophils were rare, Dorothy Reed cells were numerous. The multinucleated cells were most prominent in the sinuses just under the capsule.

The tissue showing Hodgkin's disease obtained at autopsy was uniformly made up of three main types of cells (fig 3): (1) giant cells with a maximum diameter of 40 microns, irregularly shaped, with from two to eight large vesicular nuclei which were often arranged around the periphery, occupying most of the cell, (2) smaller rounded cells with a maximum diameter of 20 microns, with a single nucleus of denser character and a wide border of deep-staining cytoplasm, (3) lymphocytes and a few plasma cells. No eosinophils were found. The mitoses, which numbered two to six per high power field, were found principally in the

10 Parsons, P. B., and Poston, M. A. *South M J* 32:7, 1939.

11 The Dominici method is as follows: Stain with eosin-orange G (0.5 Gm in 100 cc of distilled water) and counterstain with a 0.5 per cent aqueous toluidine blue.

12 The Kingoun method makes use of a stain prepared as follows: Boric fuchsin, 4 Gm; phenol crystals, 8 Gm; 95 per cent alcohol, 20 cc, and water, 100 cc. Counterstaining is done with Löffler's methylene blue solution.

13 The MacCallum method is as follows: 1. Apply the Goodpasture stain (basic fuchsin, 0.59 Gm; aniline oil, 1 cc; phenol crystals, 1 cc, and 30 per cent alcohol, 100 cc). 2. Differentiate in solution of formaldehyde U S P (40 per cent). 3. Counterstain with saturated aqueous trinitrophenol. 4. Stain with Sterling's gentian violet. 5. Stain with Gram's iodine. 6. Clear in aniline oil and xylene.

large mononuclear cells. Considerable necrosis with little fibrosis was present. Blood vessels and lymphatics in all sections contained these cells.

A dense zone of Hodgkin's tissue, having a thin layer of fibrin on the edge next to the pericardial cavity, extended into the pericardium, epicardium and myocardium. The tissue invaded the muscularis and the folds of the mucosa in the gallbladder, the muscle bundles in the sternocleidomastoid muscle and in the diaphragm, the alveoli of the lung, the mucosa of the trachea and the capsule of the thyroid. Invasion of the spleen was slight, but necroses were prominent. No invasion of the liver or bone marrow—femur, rib vertebra—was found. The femoral marrow was hyperplastic with many eosinophils in all stages of development, the red cell series and the supporting tissue were increased. At several places in the intestines the submucosa was extensively infiltrated with lymphocytes, but no multinucleated cells were seen, the mucosa was intact.

No organisms were found with acid-fast or bacterial stains.

#### COMMENT

The clinical course in the present case was strikingly similar to that of an acute infection. The pathologic observations suggest that the tissue involved in the disease had taken on malignant, invasive characteristics. This observation has been recorded previously in the literature.

Lesions in the mediastinal lymph nodes are frequently found in post-mortem examinations, but the explanation of the rare involvement of the pericardium is obscure. In most of the cases reviewed in this article, lesions in the adjacent lymphoid tissue have extended directly into the pericardium. Since appreciable quantities of lymphoid tissue which might act as primary or secondary foci for the spread of the lesions were not found distal to the anterior mediastinal or intertracheobronchial nodes, one must assume that cells reached the pericardial membrane by retrograde extension through the lymphatics, or that they entered by way of the blood.

There is said to be over the ventricles a rich subepicaudal plexus of lymphatics communicating with those of the myocardium and fusing to form two trunks parallel to the coronary arteries<sup>14</sup>. A small preaortic gland may be present as a way station in the course of the right duct, which joins the anterior mediastinal chain. Small latero-pulmonary and dorsopulmonary glands may interrupt the flow in the left trunk from the ventricle to the intertracheobronchial group. The auricles drain directly into these large groups of glands or into paraphrenic glands as a way station. Valves are rare in the lymphatics of the auricles and are most numerous in the trunks of the ventricles on the anterior surface.

The parietal pericardium drains either directly or through the paraphrenic glands into the right and left anterior mediastinal groups along with lymphatics from the thymus. On the right, an ascending trunk from this group empties into the subclavian or into the jugular vein. On the left, the group drains through the preaortic-carotid trunk into the thoracic duct. Some afferents go through the posterior mediastinal nodes into the intertracheobronchial group, which in turn empties into

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<sup>14</sup> Rouviere, H. *Anatomie des lymphatiques de l'homme*, Paris, Masson & Cie, 1932.



the thoracic duct through the paratracheal nodes. Any of the routes outlined could serve for the spread of lesions from the mediastinal nodes to the pericardium.

The lymphatics of the gallbladder are superficial to the blood vessels and anastomose with those of the liver. The lymphatics of the right side of the gallbladder drain to the lymph gland at the foramen of Winslow, which in turn sends efferents to the preaortic glands, the lateral right aortic glands, those near the superior mesenteric artery and thence into the thoracic duct. The lymphatics of the left side and inferior surface drain into the cystic gland, which is located in the curve of the neck of the gallbladder at its junction with the cystic duct. Efferents from this gland go to the gland at the foramen of Winslow and to the superior retroduodenal pancreatic group. The lymphatic drainage of the cystic and hepatic ducts is shared by both of the aforementioned glands, the common duct drains to the latter. The superior retroduodenal pancreatic and posterior duodenal pancreatic groups receive some afferents from the hepatic and common ducts, respectively. Extension along the anastomotic circulation outlined is the probable route of involvement of the biliary tract in the 2 cases reviewed.<sup>15</sup> Several other instances of lesions of Hodgkin's disease in the cystic gland without involvement of the biliary tract have been reported. No record has been found of extension from this node into the gallbladder. Why this gland and nearby lymphatics are not more frequently involved, or why lesions in the liver, which are relatively common, do not extend directly into the gallbladder, is not known.

#### SUMMARY

Eight cases of Hodgkin's disease in which pericardial lesions were proved to have occurred are reviewed, and another is reported.

Two cases of this disease in which lesions in the extrahepatic biliary tract were demonstrated are reviewed. The present case is probably unique in the invasion of the gallbladder itself.

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<sup>15</sup> Meyer<sup>4</sup> Stahl and Synwoldt<sup>9</sup>

## CHANGES IN THE INTERSTITIAL CELLS OF THE TESTES IN GULL'S DISEASE

DAVID MARINE, M D , NEW YORK

In spite of the relatively enormous literature on the pathologic aspects of myxedema in adults there are only a few specific references to changes in the gonads, although the striking difference in the sex incidence of the disease and the usual onset during the decline of active sexual life in patients of both sexes have been fully appreciated by all contributors

The more important symptoms referable to the male gonads are impotency, loss of sexual desire, decrease in the size of the testes and enlargement of the breasts

I have been unable to find any references to microscopic studies of the testes in Gull's disease, and on this account the following report of a case may be of interest

### REPORT OF A CASE

P G, aged 63, was admitted to Montefiore Hospital Dec 4, 1933, and died Sept 17, 1934 His chief complaints were gradual loss of energy, diminution of muscle strength, increased sensitivity to cold and changes in speech and facies, over a period of five years For the past six months he had had precordial pain

The man was well nourished He had acromegaloid features, slow, coarse speech and prominent lips and nose His tongue was not enlarged The upper and lower eyelids were puffy The fundi were normal The chest disclosed moderate emphysema The lungs were clear

Six months after admission the patient presented alternating diarrhea and constipation Proctoscopic examination revealed carcinoma of the rectum The tumor progressed rapidly and within two months invaded the bladder, causing cystitis and ascending infection of the kidneys

Basal metabolism tests showed a rate of — 18 per cent three days after admission and a rate of — 29 per cent three weeks later During the nine months of observation the rate fell to this level twice again, when the administration of desiccated thyroid was discontinued The red blood cell count was 4,100,000, the white cell count, 11,700, the hemoglobin content, 90 per cent The Wassermann test was negative A chemical study of the blood showed sugar, 84 mg per hundred cubic centimeters, urea, 109 mg, calcium, 10 mg, phosphorus, 37 mg, cholesterol, 207 mg Gastric analysis showed the presence of free hydrochloric acid

*Necropsy*—The anatomic diagnosis was myxedema (clinical), atrophy of the thyroid, carcinoma of the sigmoid flexure, atrophy of the interstitial cells of the testes, chromophobic adenomas of the anterior lobe of the pituitary and generalized atherosclerosis

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From the Laboratory Division, Montefiore Hospital

The heart weighed 270 Gm. The coronary arteries were sclerotic, there was partial occlusion of the left descending and left circumflex branches but no evidence of infarction. The liver weighed 1,100 Gm, was firm and cut with increased resistance. The left adrenal weighed 6.5 Gm, the right, 10 Gm. The cortex of each was well developed and of normal color. The medulla was distinct. The gastrointestinal tract was normal save for an irregular, cauliflower-like tumor,



Fig 1—Atrophic lobule of the thyroid, showing interfollicular lymphocytic infiltration and exhaustion atrophy of the follicular epithelium

beginning approximately 17 cm above and extending to within 5 cm of the anus. The testes were softer than normal. The right weighed 20 Gm, the left, 15 Gm.

The lateral lobes of the thyroid were yellowish firm bandlike masses, measuring 3 by 1 by 0.5 cm. The parathyroids appeared normal. The brain weighed 1,220 Gm. No gross abnormalities were noted. The pituitary had a normal outline and weighed 0.679 Gm.

*Microscopic Examination*—The thyroid showed generalized increase in connective tissue. The lobules were reduced to small, widely scattered nests of

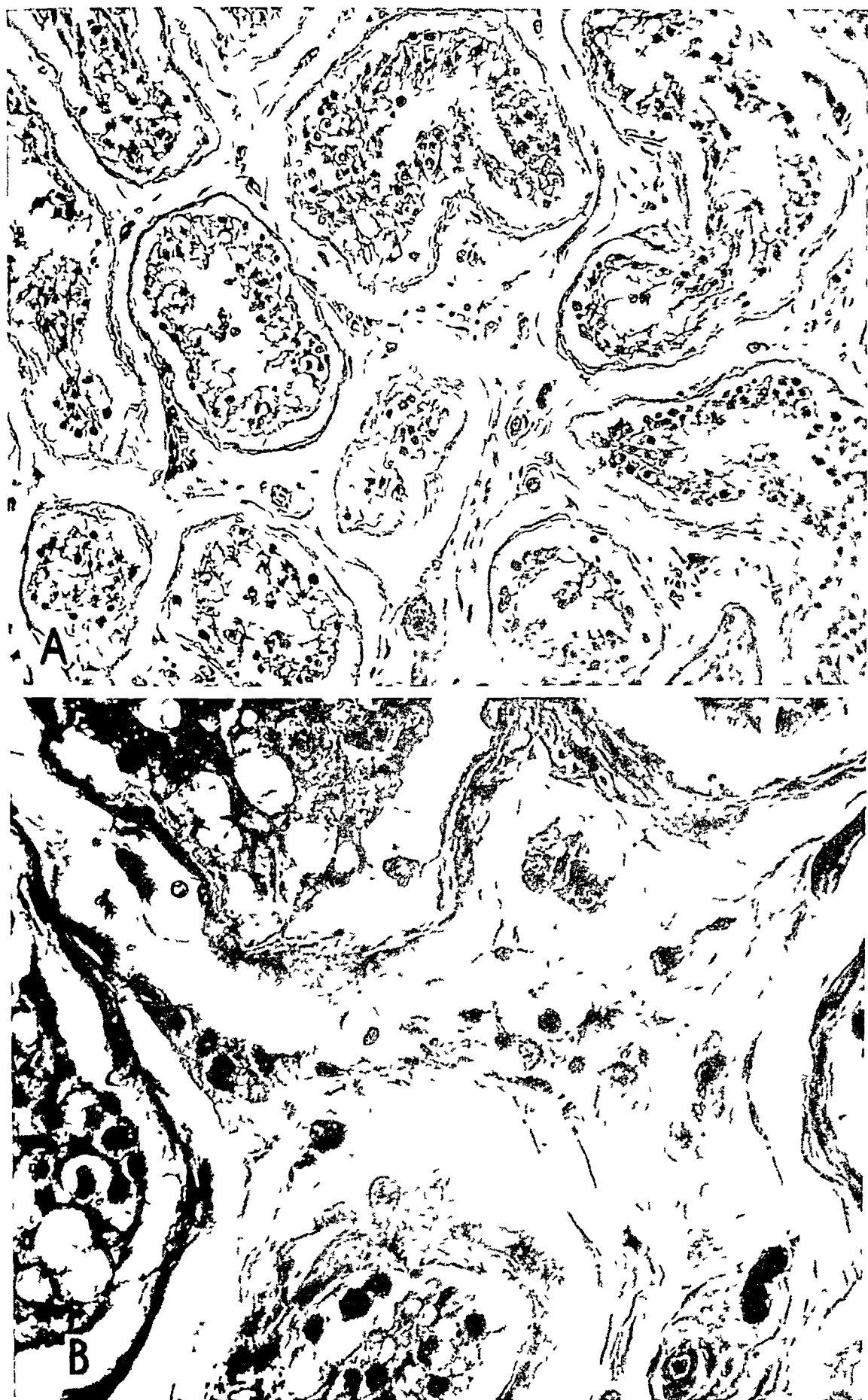


Fig 2—*A*, testicular tissue showing absence of spermatogenesis, widening of interstitial spaces, and atrophy and degeneration of interstitial cells, *B*, higher magnification of *A*

shrunk, distorted follicles, and each lobule was infiltrated by lymphocytes. The epithelial cells of the atrophic follicles were highly irregular in size and shape. Some cells contained large, distorted nuclei, in others the nuclei were small and pyknotic (fig 1).

The parathyroids were normal.

In the testes the tubules were small and widely separated. Spermatogonia were definitely reduced in number. There was no spermatogenesis. The Sertoli cells were intact. The interstitial spaces were widened, and the interstitial cells were greatly reduced in number and distorted in form. In some the nuclei had been preserved, but most of the cells were in an advanced stage of degeneration and were represented by cytoplasmic fragments or small yellowish or brownish masses of pigment (fig 2 A and B).

The pituitary revealed slight basophilic infiltration of the posterior lobe and generalized increase in the stroma of the anterior lobe. One definite and several indefinite chromophobic adenomas were noted. There was an increase in the number of cells taking a diffuse eosin stain (hematoxylin-eosin stain, Orth's fixation). With Mallory's stain, after mordanting in Zenker's fluid, the cells did not show stainable granules.

#### COMMENT

Atrophy of the testes is referred to occasionally in clinical reports of cases of Gull's disease, but I have been unable to find any reference to microscopic changes in the testes. The only detailed reports possibly related to this subject are of cases of cachexia strumipriva, by Wegelin.<sup>1</sup> In my opinion this condition is not comparable to Gull's disease because thyroidectomy during active sexual life tends to, and often does, increase the activity of the gonads, directly by removing the inhibiting effect of the thyroid hormone on the gonads and indirectly by stimulating the gonadotropic activity of the anterior lobe of the pituitary.<sup>2</sup> The increased sensitivity of the gonads to gonadotropic substances following thyroidectomy is another manifestation of this phenomenon.<sup>3</sup> One of Wegelin's patients was 47 years old and died of heart disease. A fragment of thyroid was found at necropsy. Both testes were below normal size and weight. The tubules were small, but there was definite, although greatly reduced, spermatogenesis. The interstitial spaces were widened and edematous. The interstitial cells were "not increased" and contained only a trace of pigment. Wegelin's second patient was 56 years old and died of heart disease. He had received desiccated thyroid for twenty years, beginning ten years after total thyroidectomy at the age of 16. The testes were small, and the cut surface was brownish. There was active, although greatly reduced, spermatogenesis. The interstitial cells were few and widely scattered and contained fine fat droplets, which were not doubly refractile. The hypophysis weighed 1 Gm.

1 Wegelin, C, in Henke, F and Lubarsch, O. *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1926, vol 8, p 362, *Virchows Arch f path Anat* **254** 688, 1925.

2 Marine, D, and Rosen, S H. *Am J Physiol* **121** 620, 1938.

3 Schockaert, J. *Compt rend Soc de biol* **108** 431, 1931.

It is well known that in young adult animals thyroidectomy, despite the very low metabolic rate, usually does not lead to a clinical complex resembling Gull's disease, but thyroidectomy regularly causes typical cretinism in infantile animals. In recent years somewhat the same phenomenon has been seen following subtotal thyroidectomy for exophthalmic goiter.<sup>4</sup> In some of the cases in which the metabolic rates were very low clinical signs of Gull's disease developed, while in others such signs did not, and it is possible that the different outcome may have been due in part to different levels of gonadal activity (adrenal cortices and gonads).

The significance of depressed function of the gonads in the development of myxedema has frequently been discussed, and clinical evidence has been brought forward by Curschmann,<sup>5</sup> Deusch,<sup>6</sup> Apert<sup>7</sup> and others which suggests that the decline in gonadal functions may be one of the factors underlying the subsequent failure of the thyroid. This view is based largely on clinical studies, because the anatomic changes in the ovary are difficult of interpretation and evaluation. They cannot be separated from the normal menopausal changes. There is clinical evidence, both direct and indirect, that the postmenopausal ovary may still function in such a way as to offer some protection against atrophy of the thyroid. The indirect evidence is that myxedema rarely occurs as a complication of the menopause, and the direct evidence is that cases have been reported in which myxedema developed abruptly after removal of the ovaries even when this occurred five and even ten years after the menopause. Myxedema has also been reported in males after destruction of the testes by gunshot or by tumor. The typical effect of gonadectomy on the thyroid in sexually mature animals is a temporary (one to two weeks) stimulation of the thyroid probably through the pituitary, followed by involution (as indicated by a rise in iodine [ $I_2$ ], an increase in density of colloid and flattening of the epithelium). True atrophy of the thyroid following gonadectomy has not been recorded. Anatomic changes in the testes are more easily interpreted than such changes in the ovary, and in the case reported here definite changes were observed in both the germinal epithelium and the interstitial cells. Those in the interstitial cells were much more severe—a reversal of the usual senile changes. In patients with exophthalmic goiter the interstitial cells

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4 Thompson, W. O., and Thompson, P. K. *J. Clin. Investigation* **5** 441, 1928, **6** 347, 1928. Thompson, P. K., Brailey, A. G., and Cohen, A. C. *Am. J. M. Sc.* **179** 773, 1930.

5 Curschmann, H. *Ztschr. f. d. ges. Neurol. u. Psychiat.* **41** 155, 1918.

6 Deusch, G. *Munchen med. Wchnschr.* **66** 589, 1919.

7 Apert. *Semaine med.* **28** 71, 1908.

are ordinarily well preserved even when there is advanced atrophy of the germinal epithelium

#### SUMMARY

A case of myxedema in a 63 year old man is reported in which advanced atrophy of the thyroid gland and of the interstitial cells of the testes was found. The suggestion is made that the atrophy of the interstitial cells may be etiologically important rather than coincidental.

# General Reviews

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## PROGRESS IN THE STUDY OF THE TYPHOID BACILLUS

A J WEIL, M D

L S GALL, B S

AND

S WIEDER, B S, M A

PEARL RIVER, N Y

The introduction of the exact methods of chemistry and physics into the field of microbiology and the adoption of a corresponding mental attitude have borne fruit which make the writing of a review such as this one a much more gratifying task today than it would have been fifteen years ago

It is our aim to dwell on those researches in which real progress has been made, rather than to be comprehensive. Still it is hoped that the bibliography given in the footnotes to the text is complete enough to serve as a guide to the bypaths of the subject. It includes papers available up to March 31, 1939. [In cases in which numerous publications from the same laboratory are scattered in various journals—for instance, the reports of Boivin's work—reviews published by the authors themselves are listed so as not to encumber the list with still more references.]

There are three different official classifications used for the typhoid bacillus in English speaking countries. One is *Bacterium typhosum*, used predominantly in England. Bergey's<sup>1</sup> designation *Eberthella typhosa* is increasingly followed in the United States. The third is *Salmonella typhosa*, according to the Kauffmann-White<sup>2</sup> scheme, which assigns *Eberthella typhosa* to an exact place within the *Salmonella* group.

The Kauffmann-White formula gives the predominant features of the antigenic mosaic of *E. typhosa*, namely, the somatic antigens, IX and XII, and the flagellar antigens, d in the  $\alpha$  phase and j in the  $\beta$  phase. A nonspecific H phase is not known for the typhoid bacillus. In addition to these, the scheme in its most recent shape includes the

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From the Lederle Laboratories, Inc

1 Bergey, D H. Bergey's Manual of Determinative Bacteriology, ed 4, Baltimore, Williams & Wilkins Company, 1934

2 Kaufmann, F. Ztschr f Hyg u Infektionskr 120 177, 1937



Vi antigen (For the nomenclature of typhoid receptors as used in Japan see Naito, Aoki and Tsuda<sup>3</sup>)

The flagellar, or H, antigen can be dealt with here briefly, as little new work has been done on this subject. It is generally accepted that the H antigen is of proteinic nature, but no further investigations on it have been published. Duncan<sup>4</sup> reported that H antigen is inactivated by  $\pm \frac{1}{640}$  molar hydrochloric acid in one hour at 50 C. The H antigen can be removed by repeated washing (Detre<sup>5</sup>). Pijper<sup>6</sup> showed that the flagella of the typhoid bacillus appear, on examination of the living organism, to be contorted to one or two tufts.

The opinion is now predominant that the H antibody does not contribute to antibacterial immunity (Grinnell,<sup>7</sup> Mollari, Reedy and Randall<sup>8</sup>). Whether this is true without restriction is not easy to determine. Experiences like those reported by Mudd, Lucké and Strumia<sup>9</sup> and Maltaner<sup>10</sup> seem to indicate that there are some qualities of the H antibody which may play a role in protection.

As to the somatic antigen, Furth and Landsteiner<sup>11</sup> gave conclusive evidence that its immunologic specificity is determined by a carbohydrate-like substance which contains but little nitrogen. The problem of the somatic antigen was approached by Boivin and Mesrobian<sup>12</sup> and Raistrick and Topley<sup>13</sup> independently with one member of the Salmonella group, namely, *Salmonella aertrycke*. Boivin and Mesrobian precipitated suspensions of the bacteria with trichloroacetic acid. They obtained a colloidal supernatant fluid. Further purification was achieved by precipitation with acetone, followed by dialysis. These preparations were named by the authors *antigène complet*, because they were able to demonstrate that the substance was fully antigenic. *Antigène complet* is split by acid hydrolysis into a complex carbohydrate and a fraction called by Boivin and Mesrobian *lipide*. The latter

3 Naito, T., Aoki, V., and Tsuda, D. *Ztschr. f. Hyg. u. Infektionskr.* **118** 666, 1936.

4 Duncan, J. T. *Brit. J. Exper. Path.* **16** 405, 1935.

5 Detre, L. *J. Infect. Dis.* **60** 319, 1937.

6 Pijper, A. *J. Path. & Bact.* **47** 1, 1938.

7 Grinnell, F. B. *J. Exper. Med.* **54** 577, 1931.

8 Mollari, M., Reedy, R. J., and Randall, W. A. *J. Trop. Med.* **41** 218, 1938.

9 Mudd, S., Lucke, B., and Strumia, M. *J. Immunol.* **24** 493, 1933.

10 Maltaner, F. *J. Immunol.* **26** 161, 1934.

11 Furth, G., and Landsteiner, K. (a) *J. Exper. Med.* **47** 171, 1928, (b) **49** 727, 1929.

12 (a) Boivin, A., and Mesrobian, L. *Rev. d'immunol.* **1** 553, 1935, (b) **2** 113, 1936, (c) **3** 319, 1937, (d) **4** 40 and (e) 197, 1938, (f) *Ann. Inst. Pasteur* **61** 426, 1938, (g) in *Comptes rendus du Sixième Congrès de chimie biologique*, Lyon, 1938, p. 401. (h) Mesrobian, L. *Les antigènes glucido-lipidiques des bactéries*, Thesis, Strasbourg, 1936.

13 Raistrick, H., and Topley, W. W. C. *Brit. J. Exper. Path.* **15** 119, 1934.

contains, according to these authors, fatty acids, appreciable amounts of acetic acid and small amounts of nitrogen, phosphorus and sulfur. The carbohydrate was shown to contain small amounts of nitrogen ( $\pm 2$  to 3 per cent) and up to 40 per cent reducing substances. An *antigène complet*, or *antigène glucidolipidique*, as it is also called, was shown to be present in all smooth forms of the Salmonella group and of the colon, dysentery and proteus groups. *Antigènes complets* were found to give highly specific precipitative reactions with their corresponding anti-O serums. They elicit typical anti-O antibodies when injected into animals. The polysaccharide alone as obtained by hydrolysis is a haptén. The lipid residue is not antigenic and it does not react in vitro.

Raistrick and Topley<sup>13</sup> obtained, independently from Boivin and Mesrobianu, what was evidently the same substance by a different method. They subjected washed, dried bacteria to tryptic digestion and isolated the active material by fractional alcoholic precipitation, the main fraction being obtained with 68 per cent alcohol.

Topley, Raistrick, Wilson, Stacey, Challinor and Clark<sup>14</sup> utilized the experience gained with *S. aertrycke* for isolating the corresponding substance from *E. typhi*.

More recently Henderson and Morgan<sup>15</sup> obtained very pure preparations of the "complete antigens" by extracting dried bacilli with diethylene glycol in the cold, followed by precipitation with 33 to 50 per cent acetone or 50 to 60 per cent alcohol.

The type specificities of the polysaccharides from Salmonella obtained by this method were demonstrated also by Beckwith and Morgan.<sup>16</sup> Felton and Wakeman<sup>17</sup> proposed a method for precipitating an immunizing antigen which was similar to that for preparing Felton's pneumococcic antigen. Another rather crude fractionation was described by Aoki, Ohi and Tanaka.<sup>18</sup>

The work on the somatic antigen done in France and England marks a significant step ahead. Still, one looks forward with eagerness to the results of further purification and analysis. As serologic investigation has shown, species of Salmonella often contain a plurality of somatic antigens—for instance, in *E. typhosa* are those labeled IX and

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14 Topley, W. W. C., Raistrick, H., Wilson, J., Stacey, M., Challinor, S. W., and Clark, R. O. J. *Lancet* **1** 252, 1937.

15 Henderson, D. W., and Morgan, W. T. G. *Brit. J. Exper. Path.* **19** 82, 1938.

16 Beckwith, T. D., and Morgan, H. R. *J. Bact.* **36** 28, 1938.

17 Felton, L. D., and Wakeman, F. B. *Bull. Johns Hopkins Hosp.* **60** 178, 1937.

18 Aoki, Y., Ohi, K., and Tanaka, H. *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **90** 162, 1937.

XII in the Kauffmann-White classification. These antigens cannot be separated by relative absorption with factor-specific antisera (Meyer<sup>19</sup>). It would be important to know whether these antigenic differences are anchored within one molecule or whether *antigènes complets* harbor mixtures. Relations of this kind are known for instance in the form of the Foissman antigen. (For the *antigène complet* of the Shiga bacillus see the recent paper of Meyer<sup>20</sup>.) The only example of a separation of different somatic antigens within a "complete antigen" was disclosed by the work on the V<sub>1</sub> antigen.

Before embarking on this subject, we shall relate the history of this antigen. The first description of it is credited to Felix<sup>21</sup>. The trail which led to his finding started with the old observation that typhoid strains when freshly isolated from patients often proved to be inagglutinable. (For an early American observation of this fact, see Gay and Claypole<sup>22</sup>.)

Felix and Pitt<sup>21</sup> found that such inagglutinable strains are more pathogenic for mice than are the agglutinable ones. They immunized rabbits with live cultures of an inagglutinable strain and found a new agglutinin. The antibodies produced by this antigen are of a comparatively low titer. The agglutination is of the granular type.

O and V<sub>1</sub> are antigenically strictly different. Agglutinability by V<sub>1</sub> antibody is, of course, related simply to the presence or absence of the V<sub>1</sub> antigen. On the other hand, the agglutinability of a strain by O antibody is more or less inhibited in the presence of V<sub>1</sub> antigen, and full agglutinability is present only in strains devoid of V<sub>1</sub>. This phenomenon of inhibition of O agglutination in the presence of V<sub>1</sub> antigen explains the old observation referred to. These basic observations were soon confirmed (Dyaschenko,<sup>23</sup> Almon and Stovall<sup>24</sup>).

Because of the relationship of this antigen to pathogenicity in mice and from other considerations, which we shall discuss later on, Felix called it "virulence antigen," a term soon generally abbreviated to "V<sub>1</sub> antigen." Felix and Pitt<sup>25</sup> demonstrated the antigen's independence of the presence or absence of O antigen.

Kauffman<sup>26</sup> devised the designation *V-W Formwechsel*, assigning to the V<sub>1</sub> form the symbol V and to the O form the symbol W, the intermediate forms being called V-W. Practical as this scheme is, it has not won many adherents in English-speaking countries.

19 Meyer, K. Ann Inst Pasteur **62** 281, 1939

20 Meyer, K. Compt rend Soc de biol **128** 746, 1938

21 Felix, A., and Pitt, R. M. Lancet **2** 186, 1934

22 Gay, F. P., and Claypole, E. J. Arch Int Med **12** 671, 1912

23 Dyaschenko, S. S. J Hyg **36** 108, 1936

24 Almon, L., and Stovall, W. D. J Immunol **31** 269, 1936

25 Felix, A., and Pitt, R. M. J Hyg **35** 428, 1935

26 Kauffmann, F. Ztschr f Hvg u Infektionskr **116** 617, 1935

A strain containing V<sub>1</sub> but not O antigen is rough in all its aspects. On the other hand, a strain may be devoid of V<sub>1</sub> antigen and yet be completely smooth (Felix and Pitt<sup>25</sup>). Kauffmann<sup>26</sup> and Scholtens,<sup>27</sup> while confirming Felix' observation, are not inclined to accept a strain as rough in the strict sense if it contains V<sub>1</sub>.

Topley and his co-workers,<sup>14</sup> as well as Henderson and Morgan,<sup>15</sup> Combiesco, Combiesco and Soru<sup>28</sup> and Combiesco and Combiesco<sup>29</sup> showed that it is possible to demonstrate chemical differences between the O and the V<sub>1</sub> antigen. V<sub>1</sub> antigen, in contrast to O antigen, is precipitable by phosphotungstic acid in sulfuric acid, neutral lead acetate, uranium acetate, mercuric acetate and aluminium acetate. Topley<sup>14</sup> and Henderson and Morgan<sup>15</sup> found also that their V<sub>1</sub> antigen and O antigen differ in the biuret reaction and in the ninhydrin test. Boivin and Mesrobianu<sup>30</sup> found differences in the reducing properties of O and V<sub>1</sub>. The content of carbon, hydrogen, nitrogen and phosphorus is essentially the same. Combiesco, Combiesco and Soru<sup>28</sup> reported that *antigène complet* prepared from V<sub>1</sub> strains is less filtrable through Seitz E K filters and that solutions of V<sub>1</sub> antigen are more stable than solutions of O antigen.

The V<sub>1</sub> antigen is much more labile than the O antigen. Felix, Bhatnagar and Pitt<sup>31</sup> showed that it is unstable at 100 C. According to Felix, Bhatnagar and Pitt, the immunizing quality is reduced by treatment with small amounts of formaldehyde or by moderate heat (65 C), so that the treatment of animals with such denatured material results in antibodies of poor protective properties. More intensive treatment destroys the antigenic properties entirely. It is interesting to note that formaldehydized V<sub>1</sub> antigen which is capable of evoking antibodies in rabbits is ineffective in horses. These rabbit antibodies are, however, of poor protective quality. This observation was confirmed and amplified in a recent paper by Henderson,<sup>32</sup> who found that serums made with extracts from V<sub>1</sub> strains by the diethylene glycol method, regardless of their precipitative and agglutinative titers, had poor protective quality and did not give complement fixation against whole bacilli.

27 Scholtens, R. T. Zentralbl. f. Bakt. (Abt. 1) **139** 467, 1937.

28 Combiesco, D., Combiesco, C. P., and Soru, E. Compt. rend. Soc. de biol. **126** 1081, 1937.

29 Combiesco, D., Combiesco, C. P., Dumitresco, N., and Badenski, A. *ibid.* **126** 1079, 1937.

30 Boivin, A., and Mesrobianu, L. (a) Compt. rend. Soc. de biol. **128** 5 and (b) 9, 1938, (c) Compt. rend. Acad. d. sc. **206** 1416, 1938.

31 Felix, A., Bhatnagar, S. S., and Pitt, R. M. Brit. J. Exper. Path. **15** 346, 1934.

32 Henderson, D. W. Brit. J. Exper. Path. **20** 11, 1939.

The typhoid organisms contain a number of antigenic substances which do not give evidence of their presence in the smooth forms and not always in the rough forms. Fuhr and Landsteiner<sup>11b</sup> showed that the R form contains a carbohydrate different from that of the S form. This fact was confirmed by White,<sup>33</sup> who demonstrated, in addition to a carbohydrate characteristic for the R form, the following factors:

1 The Q factor, which is extracted from organisms of the Salmonella group by hydrochloric acid in alcoholic solution. It is a true antigen common to the whole Salmonella group.

2 An antigen ascribed to the  $\rho$  form which is a hapten. (The  $\rho$  form is best described as a "loss" variant of the rough form deprived of the R hapten.) White obtained the determinant antigen by washing the organisms with hot alcohol. The R and  $\rho$  forms, moreover, contain another factor common to both.

3 An antigen called the T fraction, which is common to the Salmonella group and which he identified with the P fraction of Fuhr and Landsteiner.

Malik<sup>34</sup> recently described a "loss" variant of the R form with an antigen of its own; its relation to White's findings remains to be determined. Malik's speculation about the spatial arrangement of these "minor" antigens touches an important problem, about which but little is known.

Henderson<sup>32</sup> also reported two antigenic fractions from rough strains with complicated relations to each other and also to antigenic qualities present in smooth forms. One of these fractions seems to be responsible for a quota common to both O and V<sub>1</sub> antisera. Henderson is well aware of the similarity of these observations with those of White.

O and V<sub>1</sub> forms differ in the appearance of their colonies (Craigie and Brandon,<sup>35</sup> Giovanardi,<sup>36</sup> Malik<sup>37</sup>), colonies of V<sub>1</sub> strains being less transparent than those of O forms.

The optimal development of V<sub>1</sub> antigen depends much on proper temperature and culture medium (Felix and co-workers<sup>31</sup> Kauffmann,<sup>38</sup> Detre<sup>5</sup>).

Detre<sup>5</sup> contributed a method of selecting V forms out of V-VV cultures by adding O antisera and subculturing from the supernatant fluid, which, of course, contains the nonagglutinable organisms, rich in

33 White, P. B. *J. Path. & Bact.* **34** 325, 1931, **35** 77, 1932, **36** 65, 1933.

34 Malik, J. *Compt. rend. Soc. de biol.* **129** 802, 1938.

35 Craigie, J., and Brandon, K. F. *Canad. Pub. Health J.* **27** 165, 1936.

36 Giovanardi, A. *Zentralbl. f. Bakt. (Abt. 1)* **141** 341, 1938.

37 Malik, J. *Compt. rend. Soc. de biol.* **129** 785, 1938.

38 Kauffmann, F. *Ztschr. f. Hyg. u. Infektionskr.* **117** 778, 1936, footnote 26.

V<sub>1</sub> It seems that V<sub>1</sub>-containing micro-organisms can be obtained by suitable methods of selection from practically any strain. Both Kauffmann<sup>26</sup> and Detre<sup>5</sup> obtained, for example, V<sub>1</sub> forms from the H-901 strains.

Dwarf colonies of *E. typhosa* were regarded as irregularities for many years. However, Victorisz,<sup>39</sup> in the course of a recent study devoted to these dwarf forms, found reasons to believe that they represent the genotype of the typhoid bacillus, from which S and R forms either with or without V<sub>1</sub> antigen develop and to which they eventually revert.

The discussion about a possible transmutation of typhoid bacilli into saprophytes and vice versa came to an end after Cruickshank<sup>40</sup> refuted the story of the relation between *E. typhosa* and the chromobacterium called *Bacterium typhi flavum*.

The question of the subdivision of typhoid bacilli into types according to fermentative reactions has not attracted much attention during recent years. Kristensen<sup>41</sup> found that the fermentation or the lack of fermentation of rhamnose is a constant quality of a given strain. There is relatively little information regarding the biologic importance of differences in fermentative activities. Since Braun and Cahn-Bronner<sup>42</sup> studied the *Verwendungsstoffwechsel* (appropriative metabolism) of typhoid bacillus, this question has not obtained due attention. The few investigations on bacterial metabolism in which typhoid bacilli were involved can be properly appreciated only in connection with the general problems of this kind. For that study we refer to Stephenson's<sup>43</sup> book. Considerable help in the study of the chemistry of the micro-organisms should be obtained through the use of cultural mediums of simple or known chemical composition, apart from the theoretic and practical interest of such mediums in the field of bacterial metabolism. For instance, Gladstone<sup>44</sup> observed that the formation of V<sub>1</sub> antigen depends on the presence of dextrose but is independent of the source of nitrogen available to the bacillus.

No true exotoxin has ever been found. That means that culture filtrates from the typhoid bacillus do not contain appreciable amounts of poisonous material. Only if the bacilli are autolyzed or disrupted do toxic components of the bodies go into solution. Such solutions are called endotoxins, a rather unfortunate term, since it tends to divert

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39 Victorisz, K. Zentralbl. f. Bakt. (Abt. 1) **142** 389 and 404, 1938.

40 Cruickshank, J. C. J. Hyg. **35** 354, 1935.

41 Kristensen, M. L. J. Hyg. **38** 688, 1938.

42 Braun, H., and Cahn-Bronner, C. E. Biochem. Ztschr. **131** 226 and 272, 1922.

43 Stephenson, M. Bacterial Metabolism, ed. 2, New York, Longmans, Green & Co., 1939.

44 Gladstone, G. P. Brit. J. Exper. Path. **18** 67, 1937.

attention from the perception that the poisonous material cannot properly be called a toxin. One wonders how greatly progress has been delayed by the general acceptance of such terms. As dissolved bacterial material is thought by many to be a favorable material with which to start immunization, methods to achieve disruption with as little damage as possible to the antigenic substances have been described repeatedly during recent years. The old method of repeated alternate freezing and thawing (Luedke<sup>45</sup>) was recommended by Grasset<sup>46</sup> and Zablocki and Morzycki<sup>47</sup>. Grasset<sup>46</sup> found that it is possible to lower the toxicity of this antigen by adding formaldehyde. Just what the effect of formaldehyde is in this case is not clear, as the toxic substance is certainly not of protenic nature. Boivin<sup>48</sup> expressed the opinion that the solution of formaldehyde renders the somatic antigen less soluble, an effect which may account for the lowering of the toxicity in Grasset's *anaendotoxin*.

A very effective way of disrupting micro-organisms is to treat them with supersonic vibrations, this holds true also for typhoid bacilli (Chambers and Flosdorf<sup>49</sup>).

Boivin and Mesrobian<sup>12f</sup> found that the *antigène complet* accounts entirely for the toxicity of the bodies of typhoid bacillus. This has since been confirmed by Topley and his co-workers,<sup>14</sup> Spanedda<sup>50</sup> and Henderson and Morgan<sup>15</sup>. Spanedda<sup>51</sup> emphasized the similarity of the pathologic changes in animals killed by the *antigène complet* and the pathologic features of typhoid fever.

Delafield<sup>52</sup> observed that all these somatic antigens produce a hyperglycemic effect. Furthermore, they diminish the oxygen uptake of rabbit brain but not of muscle suspensions (Delafield and Smith<sup>53</sup>).

Dennis and Senekjian<sup>54</sup> reported on the leukocidal effect of typhoid filtrates in rabbit and human blood.

According to our experience, the extreme loss of weight of such animals as survive sublethal doses of the "complete antigen" of typhoid

45 Luedke, H. Deutsches Arch f klin Med **98** 395, 1910

46 Grasset, E. Compt rend Soc de biol **115** 1485, 1934

47 Zablocki, B., and Morzycki, J. Compt rend Soc de biol **117** 789, 1934  
Morzycki, J., and Zablocki, B. ibid **117** 792, 1934

48 Boivin, A. Ann Inst Pasteur **61** 758, 1938

49 Chambers, L. A., and Flosdorf, E. W. Proc Soc Exper Biol & Med **34** 631, 1936

50 Spanedda, A. Boll Soc ital di biol sper **11** 21, 22, 327, 931 and 933, 1936

51 Spanedda, A. Boll Soc ital di biol sper **12** 143, 1937

52 Delafield, M. E. J Path & Bact **35** 53, 1932, Brit J Exper Path **15** 130, 1934

53 Delafield, M. E., and Smith, H. A. Brit J Exper Path **17** 379, 1938

54 Dennis, E. W., and Senekjian, H. Proc Soc Exper Biol & Med **36** 61, 1937

reaches truly astounding proportions. In guinea pigs losses of 50 per cent of weight are not uncommon, and the recovery requires many weeks and even months. This recalls the familiar picture in the convalescence from typhoid fever. Further pharmacologic investigation of somatic antigens would be highly desirable.

The toxic effect of the purified somatic antigen can be prevented by active immunization either with whole bacilli or with somatic antigen, and it can be neutralized specifically by the corresponding immune serum. This point was especially studied by Boivin and Mesrobian<sup>12a</sup>. According to our experience, the demonstration of exact stoichiometric relations in the neutralizing effect is difficult because neutralization is limited to low multiples of lethal doses by the technical conditions of the experiment.

A precise determination of antigen-antibody relations is possible by means of precipitation at optimal proportions, and also by determination of antibody N (Henderson and Morgan,<sup>15</sup> our own unpublished experiments).

Preparations of "complete antigen" give powerful complement fixation with the corresponding rabbit serums but not with horse serums (unpublished experiments), an experience which parallels that of Zinsser and Parker<sup>55</sup> as regards the behavior of pneumococcal antibody.

There is an important point which requires elucidation. Boivin and Mesrobian<sup>12</sup> found that the somatic antigens of all the members of the *Salmonella* group as well as dysentery, colon and proteus bacilli are toxic to a similar degree and kill under symptoms very similar to those resulting from the administration of somatic antigen of typhoid bacilli.

These observations in animals seem to be in marked contrast to the characteristic picture of typhoid fever in man. However, this specificity is only partially real, the pathologic picture of infection with any of the various members of the *Salmonella* group, especially paratyphoid A or B is very similar to that of infection with the typhoid bacillus if the clinical features are those of typhoid fever and not of enteritis.

On the other hand, the apparent specificity of enteric infection may be purely a question of differences in the quantity of poisonous material introduced. We know that the typhoid bacillus and *Salmonella* paratyphi A, B and C can have a quite peculiar invasive power in man, in contrast to other members of the *Salmonella* group. Micro-organisms capable of existing and multiplying in the body have a greater opportunity to produce a toxic effect than has a transient invader. Therefore, for a deeper understanding of the processes in typhoid fever one should distinguish between the toxic principle and other qualities which enable the bacillus to maintain itself in the body, these qualities we cannot define precisely.



For the special case of typhoid infection the puzzle became no less intricate by reason of Felix' discovery of the V<sub>1</sub> antigen. In order to understand the situation we must first take inventory of the present knowledge of the V<sub>1</sub> antigen. It is known that this antigen is definitely a separate chemical entity. It is known that it is many times less toxic than the somatic antigen (Boivin and Mesrobian<sup>30b,c</sup>, Combesco and Combesco,<sup>29</sup> Henderson and Morgan<sup>15</sup>).

The V<sub>1</sub> antigen is practically always present in strains recently isolated from human sources, a fact which will be discussed in greater detail later on.

Felix originally was induced to connect V<sub>1</sub> antigen with "virulence" by the observation that strains containing it were more virulent for mice than those lacking it (confirmed by Kauffmann<sup>56</sup>). But in order to weigh this argument one must keep in mind that this difference between V<sub>1</sub> strains and O strains is a rather slight one, whereas an O strain, such as the famous O-901, causes lethal septicemia in white mice in doses of about 200,000,000 organisms, V<sub>1</sub> strains will have the same effect in doses of about 50,000,000 organisms.

Felix and Bhatnagar<sup>57</sup> and Kauffmann<sup>26</sup> showed that V<sub>1</sub> strains are more resistant against phagocytosis and that V<sub>1</sub> antibody is necessary to render opsonification of V<sub>1</sub> strains effective. (In the absence of V<sub>1</sub> the O antibody is the bearer of the opsonizing qualities, according to Bhatnagar<sup>58</sup>). The assumption seems not to be unreasonable that V<sub>1</sub> strains are able to overcome the 4 to 1 numerical handicap because their multiplication is less inhibited by the defenses of the body.

Moreover, the differences between O and V<sub>1</sub> strains disappear (Scholtens,<sup>27</sup> Henderson<sup>59</sup>) if infection of the mice is effected by a small number of organisms, a result which one is now in a position to achieve by the mucin method (see page 81, last paragraph). Our own experiences concur with these observations.

Felix interpreted his experimental results with active and passive immunity as indicating a relation of V<sub>1</sub> antigen to virulence. He found that treatment with vaccines made from a V<sub>1</sub> strain had a higher immunizing power than O vaccines. This observation was confirmed by Topley and his associates<sup>14</sup>. It was paralleled by the high protective value of V<sub>1</sub> antibody against infection with V<sub>1</sub> strains. Our own extensive experience leads us to believe that these differences disappear if infection is effected by the mucin method, which allows one to work with high multiples of lethal doses (10,000 to 100,000 and more), there

56 Kauffmann, F. *Ztschr f Hyg u Infektionskr* **120** 31, 1937

57 Felix, A., and Bhatnagar, S. S. *Brit J Exper Path* **16** 422, 1935

58 Bhatnagar, S. S. *Brit J Exper Path* **16** 375 1935

59 Henderson, D. W. *Brit J Exper Path* **20** 1, 1939

is no summation of the protective effect of O and V<sub>1</sub> antibody Boivin <sup>60</sup> and Boivin and Mesrobianu <sup>61</sup> recently reported the same observation

Henderson <sup>59</sup> investigated the problem of passive protection in its relation to (a) the amount and (b) the virulence of the micro-organisms He concluded that the protection was affected more by the amount than by the virulence of the micro-organisms Hence the protective action of antityphoid serums seems to resemble more an antitoxic effect than an antimetabolic one Considerations like this suggest the possibility of bringing the differences of observation to a common denominator

At the present stage of knowledge, it seems to be too early to form a definite opinion about the biologic significance of the V<sub>1</sub> antigen and the antibody against it

It may be permissible to insert here a few words regarding the efforts that have been made to find in other micro-organisms qualities similar to the V<sub>1</sub> antigen of the typhoid bacillus Felix and Pitt <sup>62</sup> expressed the belief that they had found a V<sub>1</sub> antigen in *S. paratyphi* B However, Kauffmann <sup>63</sup> held that what Felix and Pitt believed to be V<sub>1</sub> antigen is in fact the second (V) antigen of *S. paratyphi* B, which varies greatly in its quantities from strain to strain

An antigen identical with the V<sub>1</sub> antigen of the typhoid bacillus, as far as can be ascertained, was found in several strains of *S. paratyphi* C by Kauffmann <sup>26</sup> and by Rouchdi <sup>64</sup> Meyer <sup>65</sup> demonstrated recently that V<sub>1</sub> antigen from *S. paratyphi* C can be absorbed specifically by V<sub>1</sub> antiserum in contrast to the other somatic factors (see page 73, last paragraph) How far Pirofsky's <sup>66</sup> antigen of *Pasteurella avium* has biologic resemblance to the V<sub>1</sub> antigen remains to be seen The similarities mentioned by Pirofsky are (1) the fact that this antigen is also an additional somatic antigen and (2) that it is precipitable by uranium salts

We have several times given examples of the considerable help afforded by an improvement in experimentation with the typhoid bacillus This justifies our dwelling on a matter which is in itself purely technical

Rake, <sup>67</sup> following the example set by Nungester and his co-workers <sup>68</sup> and Miller <sup>69</sup> with other bacteria, found that the addition of gastric

60 Boivin, A. Compt rend Soc de biol **130** 403, 1939

61 Boivin, A., and Mesrobianu, L. Compt rend Soc de biol **130** 683, 1939

62 Felix, A., and Pitt, R. M. Brit J Exper Path **17** 81, 1936

63 Kauffmann, F. Ztschr f Hyg u Infektionskr **118** 318, 1936

64 Rouchdi, M. Compt rend Soc de biol **128** 1022, 1938

65 Meyer, K. Compt rend Soc de biol **129** 485, 1938, footnote 19

66 Pirofsky, J. Compt rend Soc de biol **127** 98 and 966, 1938

67 Rake, G. Proc Soc Exper Biol & Med **32** 1523, 1935

68 Nungester, W. J., Wolf, A. A., and Jourdonais, L. F. Proc Soc Exper Biol & Med **30** 120, 1932

69 Miller, C. P. Proc Soc Exper Biol & Med **32** 1136, 1138 and 1140, 1935

mucin to a suspension of typhoid bacilli enhances the pathogenicity of these micro-organisms to such a degree that very few are sufficient to kill (Fisk,<sup>70</sup> Buttle and others,<sup>71</sup> Henderson and Morgan,<sup>15</sup> Siler<sup>72</sup>) The mechanism of the action of mucin is unknown Our own experiences show that a relatively high concentration of mucin is required, namely, about 5 per cent, 3 per cent is inadequate, and 1 per cent is practically without effect We obtained from Dr K Meyer<sup>73</sup> the polysaccharide isolated by him from mucin and we ourselves prepared similar sugars from the neutral and the acid fraction of mucin, we found all these preparations inactive Anderson and Oag<sup>74</sup> arrived at similar results According to their findings, the protein fraction embraces the enhancing activity Whatever the case may be, mucin makes the typhoid bacillus fully virulent for the mouse and permits one to experiment under better defined conditions and in a way which is similar to true infection

The investigation of the activity of bacteriophage against the typhoid bacillus was given impetus by the finding of Sertic and Boulgakov<sup>75</sup> and of Craigie and Brandon<sup>35</sup> that the susceptibility of the organism to the action of bacteriophage is closely associated with the presence of V<sub>1</sub> antigen This has been confirmed in other countries (Scholtens,<sup>27</sup> Almon, Read and Stovall<sup>76</sup>) Scholtens demonstrated that secondary colonies from bacteriophage plaques are O forms<sup>27</sup> Paratyphoid C strains adsorb V<sub>1</sub> bacteriophages, however, they are not dissolved by them (Scholtens<sup>77</sup>) Craigie and Yen<sup>78</sup> recently found that a number of types of bacteriophage against V<sub>1</sub> forms exist These are distinguished by their different antigenic qualities and by their action on different strains of the organisms It is therefore likely that the typhoid bacillus exists in different types whose fine differences are at present recognizable only by means of their different susceptibility to the different bacteriophages Craigie and Yen<sup>78</sup> were able to demonstrate the relative uniformity of strains derived from a common epidemiologic source by means of their susceptibility or resistance to different types of bacteriophage

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70 Fisk, R T Proc Soc Exper Biol & Med **38** 659, 1938

71 Buttle, G A H, Parish, H J, McLeod, M, and Stephenson, D Lancet **1** 681, 1937

72 Siler, J F Mil Surgeon **80** 91, 1937

73 Meyer, K, Smyth, E M, and Palmer, J W J Biol Chem **119** 73, 1937

74 Anderson, C G, and Oag, R K Brit J Exper Path **20** 25, 1939

75 Sertic, V, and Boulgakov, N A Compt rend Soc de biol **122** 35, 1936

76 Almon, L, Read, J, and Stovall, W D Am J Pub Health **27** 357, 1937

77 Scholtens, R T J Hyg **37** 315, 1937

78 Craigie, J, and Yen, C H Canad Pub Health J **29** 448 and 484, 1938  
Craigie, J ibid **30** 37, 1939

Scholtens<sup>79</sup> demonstrated physical differences of various antigenic types of typhoid bacilli by the method of acid agglutination, V<sub>1</sub> forms have maximal agglutinability at a  $p_H$  of about 2.2, whereas the H antigen is agglutinated at about  $p_H$  4.6. These findings are in accordance with observations of Malek<sup>80</sup>.

At this place it may be mentioned that some observations of Malek's<sup>80c</sup> as well as of Giovanardi's<sup>81</sup> point to a connection between V<sub>1</sub> and H antigen, which seems to merit further attention. Possibly, Henderson's findings on complement fixation of live V<sub>1</sub>-H bacillus could be interpreted in a similar sense.<sup>82</sup>

Rough and smooth forms were shown by Hirsch<sup>83</sup> and Giovanardi<sup>81</sup> to be different in their flocculability by acriflavine hydrochloride, this is in correlation with the long known difference in stability in salt solutions.

The evidence accumulated since Felix's<sup>21</sup> first description shows that practically all strains freshly isolated from cases of human infection contain V<sub>1</sub>. The data compiled by different authors (Felix, Krikorian, and Reitler,<sup>84</sup> Kauffmann,<sup>38</sup> Bhatnagar and others,<sup>85</sup> Malek,<sup>87</sup> Onetto and others,<sup>86</sup> Craigie and Brandon,<sup>35</sup> Almon, Read and Stovall,<sup>76</sup> Horgan,<sup>87</sup> Welch and Mickle<sup>88</sup>) differ only in the relative frequency of V<sub>1</sub> forms and intermediate forms (V-W forms of Kauffmann).

It seems increasingly advisable to add V<sub>1</sub> antiserum to the usual array of H and O antisera for routine identification of typhoid bacilli isolated from feces, blood and other material. The preparation of such serum was first feasible only by absorbing serum containing H, O and V<sub>1</sub> antibody with an OH strain—for instance, H-901. Felix and Pitt<sup>25</sup> then found more or less rough strains of the type V<sub>1</sub>H, and recently Bhatnagar<sup>89</sup> described a strain of this type, which he obtained from Kauffmann, he found it to be so poor in flagellar antigen that antiserum prepared with it is practically pure V<sub>1</sub> antiserum. The availability of such strains promises to facilitate greatly the preparation of V<sub>1</sub> antiserum.

79 Scholtens, R. T. *J. Hyg.* **38** 273, 1938.

80 (a) Malek, I. *Compt. rend. Soc. de biol.* **129** 788, (b) 795 and (c) 797, 1938.

81 Giovanardi, A. *Ztschr. f. Hyg. u. Infektionskr.* **120** 273, 1937.

82 Henderson,<sup>32</sup> table 3.

83 Hirsch, W. *J. Path. & Bact.* **44** 349, 1937.

84 Felix, A., Krikorian, K. S., and Reitler, R. *J. Hyg.* **35** 421, 1935.

85 Bhatnagar, S. S., Speechly, C. G. J., and Singh, M. *J. Hyg.* **38** 663, 1938.

86 Onetto, E., Levton, G., and Luna, N. *Rev. d. Inst. bact. de Chile* **6** 55, 1937.

87 Horgan, E. S. *J. Hyg.* **36** 368, 1936.

88 Welch, H., and Mickle, F. L. *Am. J. Pub. Health* **27** 351, 1937.

89 Bhatnagar, S. S. *Brit. M. J.* **2** 1195, 1938. Bhatnagar and others<sup>85</sup>.

Felix, Krikorian and Reitler<sup>84</sup> found V<sub>1</sub> antibody in serum from patients with typhoid fever and in serum from typhoid bacillus carriers. Men behave like animals inasmuch as they also generally show low titers of antibodies for V<sub>1</sub> antigen (confirmed by Almon, Read and Stovall<sup>76</sup>). On the other hand, it seems that titers as low as 1:10 are significant. Whereas V<sub>1</sub> antibodies are observed in the serums of acutely ill persons and convalescents only irregularly, they are found rather constantly present in the serum of carriers (Felix,<sup>90</sup> Bhatnagar,<sup>89</sup> Lewin<sup>91</sup>). This is of considerable practical interest, as it furnishes an additional diagnostic aid in the detection of carriers.

In this connection, it should be mentioned that carriers harbor, as a rule, smooth strains more or less rich in V<sub>1</sub> and not R strains, as one would be inclined to suppose. The transition from S into R forms in typhoid bacilli seems to be ruled by influences quite other than those which rule such a transition in, for instance, the pneumococci. One may even ask whether R variants of typhoid bacillus are really "physiologic" forms or whether they merely appear under the artificial conditions of culture.

Active immunization against typhoid fever underwent an extensive test in the Italian army during the Abyssinian campaign (Castellani<sup>92</sup>). The value of this experience is somewhat impaired by the fact that the predominance of diseases against which no method of active immunization is available was similarly diminished by methods of general sanitation. Nevertheless, it is a feat that typhoid fever was practically not existent in the Italian army.

The question of the best method of active immunization has been investigated from a variety of aspects. Since Grinnell's<sup>93</sup> study established the importance of "smoothness" for the immunizing effect, general consent exists over the necessity of using only strictly smooth strains for the preparation of vaccine (see, for instance, the articles by Brown<sup>94</sup> and Dennis and Berberian<sup>95</sup>). Dennis and Senekjian<sup>96</sup> suggested the measurement of opsonic activity of human blood as a method of gauging immunity. Mollari, Reedy and Randall<sup>8</sup> found considerable protective qualities against infection of the mouse with *Salmonella enteritidis*, which contains the somatic factor "IX" as does the typhoid bacillus in human serum after typhoid vaccination.

90 Felix, A. *Lancet* **2** 738, 1938.

91 Lewin, W. *Typhoid Fever on the Witwatersrand. Bacteriological Aspects, Serological Diagnosis, Specific Prophylaxis and Specific Treatment*, Publication 41, South African Institute for Medical Research, Johannesburg, 1938, vol. 7, p. 413.

92 Castellani, A. *Mil Surgeon* **81** 1, 1937.

93 Grinnell, F. B. *J Exper Med* **56** 907, 1932.

94 Brown, M. H. *Canad Pub Health J* **27** 170, 1936.

95 Dennis, E. W., and Berberian, D. A. *Am J Hyg* **20** 469, 1934.

96 Dennis, E. W., and Senekjian, H. *Am J Hyg* **26** 11, 1937.

A detailed description of the methods used by the United States Army Medical School for the preparation of vaccines was given by Holt and Hitchens<sup>97</sup>. The United States Army Medical School also conducted investigations to determine the most appropriate strain (Siler<sup>98</sup>). They used protection in mice as a criterion in the evaluation of immunizing activity. Of special importance seems to be the observation by Siler and Dunham<sup>99</sup> that for revaccination a single small intradermal injection is as effective as the repeated subcutaneous ones generally used.

Subcutaneous administration of formaldehyde-killed suspensions of typhoid bacilli is still the predominant method. Tuft<sup>100</sup> was the first to recommend intradermal vaccination against typhoid fever. He found that one seventh to one tenth of the usual subcutaneous dose applied intradermally was sufficient to evoke a satisfactory response, and he reported considerably fewer undesirable reactions. Perry<sup>101</sup> reported the same results in a more recent paper.

The oral administration of vaccine (typhoral [Lilly] preceded by bile salts) has met with approval (Crimm and Short,<sup>102</sup> Moor and Brown<sup>103</sup>) and criticism (Dennis and Beiberian,<sup>95</sup> Lewin<sup>91</sup>).

Stuart and Krikorian<sup>104</sup> found deterioration of the immunizing effect with aging of vaccines. This observation may explain why occasionally vaccines made from local strains were found to be more effective than those obtained from abroad (Grasset<sup>105</sup>).

Grasset and Lewin<sup>106</sup> used successfully alum precipitated, formaldehydized lysates ("anatoxin").

Bhatnagar and co-workers<sup>107</sup> advised not more than a one year interval between the first and the second vaccination. This opinion may be conditioned by the special necessities of India, where this work was done. The length of time during which antibodies remain in the serum is set forth in Siler's statistics<sup>99</sup>.

As far as one knows at the present time, the usual vaccines do not evoke V<sub>1</sub> antibody in man (Bhatnagar and others,<sup>107</sup> Siler and Dunham<sup>99</sup>). Hence man in his ability to form V<sub>1</sub> antibody seems to follow the pattern of horses rather than that of rabbits, which are able to

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97 Holt, R. L., and Hitchens, A. P. *Pub Health Rep* **52** 829, 1937

98 Siler, J. F. *Am J Pub Health* **26** 219, 1936

99 Siler, J. F., and Dunham, G. C. *Am J Pub Health* **29** 95, 1939

100 Tuft, L. *J Infect Dis* **50** 98, 1932

101 Perry, R. M. *Am J Hyg* **26** 388, 1937

102 Crimm, P. D., and Short, D. M. *Am J M Sc* **196** 826, 1938

103 Moor, H. D., and Brown, J. L. *J Lab & Clin Med* **22** 1216, 1938

104 Stuart, G., and Krikorian, K. S. *Lancet* **2** 645, 1934

105 Grasset, E. *J M A South Africa* **4** 380, 1930

106 Grasset, E., and Lewin, W. *Compt rend Soc de biol* **125** 979, 1937

107 Bhatnagar, S. S., Freeman, J. F., and Dhilon, J. C. S. *Indian J M Research* **24** 597, 1937

respond to formaldehydized V<sub>1</sub> antigen. However, in making such a statement one must not forget that human beings receive only small doses of vaccine in comparison with the doses used in the hyperimmunization of animals. Thus, lacking the means of evoking V<sub>1</sub> antibody in man, one must regard the question whether V<sub>1</sub> antibody would improve the protection by vaccination as an academic one. Whether the use of purified antigens in the immunization of man would render it possible to obtain V<sub>1</sub> antibodies by vaccination is a still unsolved problem. As pointed out earlier in this article, "complete antigens" are fully antigenic in animals, however, the stimulative effect is not quite as satisfactory as that of vaccines (Felix and Petrie,<sup>108</sup> our own unpublished experiences).

According to Maccolini,<sup>109</sup> addition of alum, hydrous wool fat and other substances used to enhance the immunizing properties of true toxin has no effect if the substance is added to "complete antigens."

For the treatment of typhoid fever, vaccines were used only in a few places during recent years. Frowley<sup>110</sup> reported on his experiences with lysates of typhoid bacilli for therapeutic use, following recommendations originating from Caronia. These lysates are prepared by adding human blood to the cultures and allowing it to exercise its bacteriolytic power. Lewin<sup>91</sup> used "endotoxoid" vaccines, according to Grasset<sup>46</sup> (formaldehydized typhoid bacilli lysates).

Lewin<sup>91</sup> reported favorably on the use of serum prepared by immunization of horses with the formaldehydized autolysates of Grasset,<sup>46</sup> thus confirming Grasset's observation (Gory and Grasset,<sup>111</sup> Grasset and Lewin<sup>112</sup>).

Even more encouraging are reports on the therapeutic effect of a preparation produced by Felix<sup>113</sup> at the Lister Institute. Felix and Petrie<sup>108</sup> recently described in detail methods for the preparation of such serum, and Felix,<sup>114</sup> his methods of assay.<sup>114b</sup> The horses are treated with vaccines made from O strains killed with alcohol (Felix found that such preparations preserve the antigenicity better than the usual formaldehydized vaccines) and subsequently with live rough V<sub>1</sub> strains for the stimulation of an additional output of V<sub>1</sub> antibody.

108 Felix, A., and Petrie, G. F. *J. Hyg.* **38** 673, 1938.

109 Maccolini, R. *Boll. Soc. ital. di biol. sper.* **13** 900 and 1079, 1938.

110 Frowley, J. M. *California & West Med.* **48** 415, 1938.

111 Gory, M., and Grasset, E. *Compt. rend. Soc. de biol.* **98** 435, 1928.

112 Grasset, E., and Lewin, W. *Brit. J. Exper. Path.* **18** 460, 1937.

113 Felix, A. *Lancet* **1** 799, 1935.

114 Felix, A. *J. Hyg.* **38** 750, 1938.

114b Recommended provisionally as standard by the Health Organization of the League of Nations (Report on the Meeting of Serologists of the Permanent Commission on Biological Standardisation, *Bull. Health Organ., League of Nations* **7** 701, 1938).

The treatment with live bacilli seems to be well tolerated by the horses. There is only one report (Petrie<sup>115</sup>) of bacilluria in a horse after such treatment, but even this horse did not have positive blood cultures. The serum is concentrated by salting out with ammonium sulfate. Reports are uniformly favorable as to the effect of the serum on mortality, fever, toxic symptoms and duration of the disease (Felix,<sup>113</sup> McSweeney,<sup>116</sup> Robertson and Yu,<sup>117</sup> Lewin,<sup>91</sup> Cookson and Facey<sup>118</sup>)

It is generally agreed that the earlier during the course of the infection serum treatment is started the greater is the expectancy of a satisfactory result.

The prophylactic use of such serum was attempted recently during a local epidemic in England (Fenton and Hay<sup>119</sup>). The authors have abstained from any definite statement as to its usefulness, because of the small number of persons treated. Their experience seems to justify their recommendation of further trials in that direction. Topley<sup>120</sup> discussed the advisability of combining prophylactic application of serum with active immunization by vaccine in cases in which there is immediate danger of infection, in such cases the passive immunization could possibly bridge the interval between the beginning of active immunization and the point at which actual active immunity is achieved.

Shwartzman, Baehr and Hollingsworth<sup>121</sup> reported on a series of 80 cases of typhoid fever in which treatment was carried on with a serum obtained from horses immunized with typhoid filtrates. The inhibitive effect on the Shwartzman phenomenon was used as the experimental measure of the activity of the serum (Shwartzman<sup>122</sup>).

A study of Rosenheim<sup>123</sup> on the action of enzymes may be mentioned here as a possible first step in the direction of future purification of typhoid antiserum parallel to recent methods of treatment of antitoxins (Weil, Parfentjev and Bowman<sup>124</sup>). Rosenheim found O antibodies easily destroyable by pepsin, trypsin and papain. H antibodies

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115 Petrie, G. F. *J. Path. & Bact.* **42** 75, 1936

116 McSweeney, C. J. *Brit. M. J.* **2** 1118, 1937

117 Robertson, R. C., and Yu, H. *Brit. M. J.* **2** 1138, 1936

118 Cookson, H., and Facey, R. V. *Brit. M. J.* **1** 1009, 1937

119 Fenton, Y., and Hay, C. P. *Brit. M. J.* **1** 1090, 1938

120 Topley, W. W. C. *Lancet* **1** 181, 1938

121 Shwartzman, G., Baehr, G., and Hollingsworth, W. Y. *Arch. Int. Med.* **58** 799, 1936

122 Shwartzman, G. *Phenomenon of Local Tissue Reactivity and Its Immunological, Pathological and Clinical Significance*, New York, Paul B. Hoeber, Inc., 1937

123 Rosenheim, A. H. *Biochem. J.* **31** 54, 1937

124 Weil, A. J., Parfentjev, I. A., and Bowman, K. L. *J. Immunol.* **35** 399, 1938



obtained from horses immunized for several weeks behave in much the same way. However, serum from horses immunized for a longer period were found to contain an H antibody considerably more resistant to the action of pepsin and trypsin—an interesting example of variability of antibodies.

Asheshov, Wilson and Topley<sup>125</sup> obtained a remarkable therapeutic effect with bacteriophage in experimental typhoid infection of mice when microbes and bacteriophage were injected simultaneously. The effect was considerably lessened if bacteriophage was introduced four hours *post infectionem*. The results were confirmed by Fisk<sup>70</sup>. One will recall the French endeavors of treating typhoid fever with bacteriophage (d'Herelle<sup>126</sup>).

As in almost every other disease, sulfanilamide has been tried in the treatment of typhoid fever. Buttle<sup>127</sup> found some activity of the drug in experimental infection of mice. McIntosh and Whitby<sup>128</sup> found that agglutinins developed in mice infected with sublethal doses of the Vi strain the same as in the controls. There have since been a few clinical trials (Diefenbach and Yuskis,<sup>129</sup> Barum<sup>130</sup>). The small number of patients treated does not allow one to draw conclusions as to the effect of the drug.

Our insight into the causes which make the typhoid bacillus (in contrast to so many other related micro-organisms) the etiologic agent of a disease strictly confined to man and characteristic in its pathologic effect is poor. A wide field for the experimental pathologist is still open here. We have clues in different directions which could help in starting an analysis by methods of experimentation. Oerskov and Kauffmann<sup>131</sup> studied the manner of infection with different members of the Salmonella group in mice. Within the Salmonella group one finds all types of infectivity for mice, infection with the typhoid bacillus requires a very large number of organisms even when these are introduced parenterally, unless helped by the addition of mucin, *S. paratyphi B* is lethal when introduced parenterally in moderate numbers (thousands), *Salmonella breslau* introduced orally gives rise to a general infection. Oerskov demonstrated that all these bacteria are able to penetrate the enteric wall and yet they do not progress farther than the regional lymph glands, with the exception of the *Breslau* bacilli, which migrate through

125 Asheshov, J. N., Wilson, J., and Topley, W. W. C. *Lancet* **1** 319, 1937.

126 d'Herelle, F. *The Bacteriophage and Its Behavior*, translated by G. H. Smith, Baltimore, Williams & Wilkins Company, 1926.

127 Buttle, G. A. H. *Lancet* **2** 1076, 1937. Buttle and others<sup>71</sup>.

128 McIntosh, J., and Whitby, L. E. H. *Lancet* **1** 431, 1939.

129 Diefenbach, W. E., and Yuskis, A. S. *California & West Med* **49** 146, 1938.

130 Barum, R. *Lancet* **2** 964, 1937.

131 Oerskov, J., and Kauffmann, F. *J. Hyg.* **36** 5141, 1936.

the lymph glands into the general circulation and are thence carried into the organs, where they multiply and reenter the blood stream when multiplication has progressed further, finally the organisms reappear in the feces (if death has not intervened). There is good reason to believe that human typhoid fever is essentially similar to the Breslau infection in the mouse.

But with this analogy knowledge comes to an end. All the data mentioned in this review do not give a satisfactory explanation of the underlying differences between the typhoid bacillus and its harmless and often commensal relatives.

A method of analysis which may help to solve this problem is indicated by the recent work of Goodpasture and Anderson<sup>132</sup>. These investigators started by observing the processes which take place when the chorioallantoic membrane of the chick embryo is infected with the typhoid bacillus, they demonstrated that the reaction differs greatly from that to other micro-organisms. They found that the growth of the typhoid bacillus occurs preponderantly within the body of certain cells, which they identified as plasma cells. (It would be interesting to know with what variant of the typhoid bacillus these experiments were done.) In a subsequent study, Goodpasture<sup>133</sup> found a quite similar situation in human material. He demonstrated that here also the typhoid bacillus is taken up into the bodies of the plasma cells and that it multiplies there. The destruction of typhoid bacilli takes place only if the plasma cells filled with bacilli or the free micro-organisms are phagocytosed by macrophages. These observations show how careful one should be with the equation phagocytosis = defense reaction. Goodpasture has not yet included other gram-negative rods of the colon bacillus-dysentery bacillus-Salmonella group in his experiments, therefore the question is still open as to whether the typhoid bacillus occupies an exceptional position within this group.

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132 Goodpasture, E. W., and Anderson, K. *Am J Path* **13** 149, 1937.

133 Goodpasture, E. W. *Am J Path* **13** 175, 1937.

## Notes and News

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**University News, Promotions, Resignations, Appointments, Deaths, Etc**—The American Society for Experimental Pathology has elected E W Goodpasture, president, Shields Warren, vice president, Paul R Cannon, secretary-treasurer. The next meeting will be held in New Orleans in March 1940, in conjunction with the Federation of American Societies for Experimental Biology.

On account of Robert Moore assuming the professorship of pathology in Washington University School of Medicine, St Louis, Charles T Olcott has been elected secretary of the New York Pathological Society.

**Institute on Blood Diseases**—The University of Wisconsin Medical School is to conduct an institute for the consideration of blood and blood-forming organs, Sept 4 to 6, 1939. The program will include reading of papers and round table discussions by European and American workers in the field of hematology. Formal papers will be presented by the following:

L J Witts, Oxford, England "Anemias Due to Iron Deficiency"

Cecil J Watson, Minneapolis "The Porphyrins and Diseases of the Blood"

Cornelius P Rhoads, New York "Aplastic Anemia"

E Meulengracht, Copenhagen "Some Etiological Factors in Pernicious Anemia and Related Macrocytic Anemias"

Harry Eagle, Baltimore "The Coagulation of Blood"

George R Minot, Boston "Anemias of Nutritional Deficiency"

Russell L Haden, Cleveland "The Nature of the Hemolytic Anemias"

Jacob Furth, New York "Experimental Leukemia"

Claude E Forkner, New York "Monocytic Leukemia and Aleukocythemic Leukemia"

Edward B Krumbhaar, Philadelphia "Hodgkin's Disease"

Louis K Diamond, Boston "The Erythroblastic Anemias"

Edwin E Osgood, Portland, Ore "Marrow Cultures"

Charles A Doan, Columbus, Ohio "The Reticulo-Endothelial System"

Hal Downey, Minneapolis "Infectious Mononucleosis"

Paul Reznikoff, New York "Polycythemia"

Physicians and others who are interested are cordially invited. A detailed program may be obtained by addressing Ovid O Meyer, chairman of the Program Committee, University of Wisconsin Medical School, Madison, Wis.

**Society News**—The annual meeting of the Biological Photographic Association will be held September 14-16, at the Mellon Institute for Industrial Research, Pittsburgh. The program will be of interest to scientific photographers, scientists who use photography as an aid in their work, teachers in the biologic fields, technical experts and serious amateurs. It will include discussions of motion picture and still photography, photomicrography, color and monochrome films, processing and other procedures in the field of scientific illustrating. Up-to-date equipment will be shown in the technical exhibit, and the print salon will display the work of many of the leading biologic photographers here and abroad. The *Biological Photographic Association Journal* is published quarterly, constituting a volume of about 250 pages, which is furnished free to members. Membership privileges include the use of an authoritative question and answer service and the right to borrow loan albums and exhibits of scientific prints for study and display. For further information, write to the Secretary of the Biological Photographic Association, University Office, Magee Hospital, Pittsburgh.

# Abstracts from Current Literature

TO SAVE SPACE THE ORIGINAL TITLES OF ABSTRACTED ARTICLES SOMETIMES  
ARE SHORTENED

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## Experimental Pathology and Pathologic Physiology

REGENERATION OF THE ADRENAL GLAND FOLLOWING ENUCLEATION D J INGLE  
and G M HIGGINS, Am J M Sc **196** 232, 1938

When one adrenal gland is enucleated and the other removed, adequate cortical regeneration proceeds within the enucleated gland. When one adrenal gland is enucleated and the other left intact, there is no, or only very slight, regeneration within the enucleated gland. The presence of the functioning gland suppresses regeneration within the enucleated gland. If a normal adrenal gland is removed eight weeks after enucleation of the other gland, regeneration within the enucleated gland is then stimulated to occur in the normal way. Daily oral administration of 5 cc of an extract of adrenal cortex completely suppresses regeneration within an enucleated gland even when the other adrenal gland has been removed. Subcutaneous administration of amounts of the extract comparable to those given by mouth will restrict but not completely suppress cortical regeneration within the enucleated gland. Hypophysectomy coupled with enucleation of both adrenal glands completely suppresses regeneration of the cortical tissue. In the presence of half of the anterior lobe of the pituitary there is normal regeneration in the enucleated adrenal glands.

FROM AUTHORS' SUMMARY

EFFECT OF SEX HORMONES ON THE RENAL EXCRETION OF ELECTROLYTES G W  
THORN and L L ENGEL, J Exper Med **68** 299, 1938

In normal male dogs subcutaneous injections of progesterone, estrone (1 e, theelin), alpha-estradiol or testosterone propionate were followed by decreases in the sodium and chloride excreted in the urine. Marked differences were noted in the potency of these compounds and in the duration of the effects following single subcutaneous injections. The injection of estrone, alpha-estradiol or testosterone propionate was followed by a decrease in the inorganic phosphorus and total nitrogen excreted in the urine. On the day of injection of progesterone, estrone, alpha-estradiol or testosterone propionate a slight increase in the potassium excreted in the urine frequently followed. Experiments on adrenalectomized dogs indicated that the effect of the gonadotropic substances on the renal excretion of electrolytes was not necessarily mediated through the adrenal gland. With the possible exception of progesterone, none of the compounds studied was effective in prolonging the life of adrenalectomized male dogs.

FROM AUTHORS' SUMMARY

EXPERIMENTAL CHOLECYSTITIS H G ARONSOHN and E ANDREWS, Surg, Gynec  
& Obst **66** 748, 1938

By retrograde introduction of a fine ureteral catheter through an opening in the common duct and subsequent plastic repair of the latter a nontraumatic technic was devised for introducing substances into the dog's gallbladder. Most strains of bacteria, even when injected in overwhelming numbers, did not cause cholecystitis unless trauma was an added factor. Carbon dioxide and chlorine were absorbed rapidly from the normal gallbladder, while phosphorus was excreted, but an unknown anion accounted for much of the acidification. Egg albumin injected into a dog's gallbladder set up acute inflammation. An injec-

tion of bile salts produced changes which on gross and microscopic examination closely resembled those found in human cholecystitis. The differences in the activity of different fractions of bile were mainly quantitative, desoxycholic acid proved to be the most effective fraction, causing gangrene of the gallbladder and death of the animal in many instances, while purified and hydrolyzed bile salts were somewhat weaker. Glycocholic acid caused a marked reaction only in the higher concentrations. Deproteinized dog or ox bile left the wall of the dog's gallbladder unchanged. No protective action of protein, as described in the literature, could be demonstrated. Replacement of normal dog bile by bile concentrated to about half its volume had a marked effect. Changes in the hydrogen ion concentration of bile rarely brought about a reaction in the wall of the gallbladder unless they were extreme, less than 3 and greater than 10. Such ranges are not likely to arise in man. The toxic effect of bile salts is not due to a change in their hydrogen ion concentration. It is possible to produce experimentally an allergic condition in the gallbladder. Intravenously injected egg albumin caused edema of the gallbladder wall in previously sensitized dogs, while it caused no reaction in control animals. A pronounced edema was produced by intravenously injected bile salts. This was observed to occur within three minutes after the injection. It is suggested that a temporary increase in the concentration of bile salts in the human gallbladder brings about cholecystitis.

FROM AUTHORS' SUMMARY (WARREN C HUNTER)

**BONE CHANGES IN EXPERIMENTAL HYPERTHYROIDISM AND IN EXOPHTHALMIC GOITER** J MARTOS, Beitr z path Anat u z allg Path **100** 293, 1938

Experimental hyperthyroidism in rabbits and guinea pigs, induced by multiple subcutaneous injections of thyroxin, brought about osteoclastic lacunar atrophy, most pronounced in the tibia and mandibles. Systematic investigation of the skeletal systems of 12 persons with exophthalmic goiter revealed nonspecific osteoclastic lacunar atrophy in eleven, most intense in the femur and accompanied by proliferation of a fiber-rich connective tissue in the secondarily dilated haversian canals. In places these changes completely mimicked generalized fibrous osteitis. The differences between the bony lesions in man and experimental animals were essentially quantitative, the lesions in the former were more intense because of their longer duration. No anatomic changes were present in the parathyroid glands.

The author agrees with Askanazy that thyroxin is the immediate etiologic factor, and the atrophy of bone is the result of hyperfunction of the thyroid gland.

R J LEBOWICH

### Pathologic Anatomy

**VARIETIES OF SINGLE CORONARY ARTERY IN MAN, OCCURRING AS ISOLATED CARDIAC ANOMALIES** E B KRUMBHAAR and W E EHRLICH, Am J M Sc **196** 407, 1938

Two cases of absence of a coronary artery are reported, in both of which the observation was incidental at autopsy, the anomaly apparently having caused no damage to the myocardium. In the first case a large left coronary artery continued around the auricular-ventricular groove to the anterior surface of the right ventricle, giving off branches that corresponded to those normally given off by both arteries (Hyrtl's type of absence of the coronary artery). In the second case a large right coronary artery supplied most of the heart with conventional branches. Near its origin, however, it gave off one large anomalous branch, which passed behind the aorta to supply a good part of the left ventricle, and another, to the ventricular septum. The possibility must be considered that the former of these represented a true left coronary artery arising from a misplaced anlage, though the similar cases of Bochdalek and Sanes make this very unlikely. Other cases of absence of a coronary artery or possibly of misplacement of the anlage are tabulated, all but 3 of which fall into two groups corresponding to the types described here.

FROM AUTHORS' SUMMARY

BLOOD VESSELS IN LUNGS R D WRIGHT, J Path & Bact **47** 489, 1938

In lungs from human cadavers the pulmonary and bronchial arteries and their branches have been injected to show their distribution in pathologic lesions. Proliferative tuberculous lesions and actively growing chorionic carcinomas are avascular. The fibrous scars of tuberculous and silicotic lesions have vessels injected from the bronchial artery. The vessels which develop in fibrosarcomas growing in the lung are injected from the bronchial arteries. If in the adult lung there develops a tissue which is usually supplied from the systemic arterial circulation, the vessels which grow with it are injected from the bronchial artery. This development of new vessels from the systemic arteries may be closely linked with the excitation of collagenous tissue to further development, irrespective of the nature of the agent causing the collagenous proliferation.

FROM AUTHOR'S SUMMARY

GENETICS OF TRANSPOSITION OF VISCERA E A COCKAYNE, Quart J Med **7** 479, 1938

The thoracic and abdominal organs are at first median and symmetric. Transposition of the viscera consists in formation of a sinistral instead of a dextral spiral. Complete transposition of the viscera is inherited as a recessive and is determined by a single autosomal gene. Proofs of this are found in its familial incidence and general distribution within a family, its occurrence in both twins of a monozygotic pair and the high percentage of marriages between first cousins that give rise to it (6 in 52 consecutive fraternities). The ratio of affected to normal sibs in the fraternities, so far as this can be ascertained, agrees with that expected of a recessive character. An exceptional case of monozygotic twins, one normal and the other with transposed viscera, has been recorded. Either somatic mutation or the loss of an autosomal chromosome would account for this. Most authors state that there is a great excess of males with the condition, but in my series the excess of males is small. The incidence of congenital morbus cordis is abnormally high in complete transposition of the viscera, and, according to Kartagener, bronchiectasis is commoner than in normal persons. There are three forms of partial transposition of the viscera that affecting both thoracic and abdominal organs, that affecting only the thoracic organs and that affecting only the abdominal organs. Their relationship to one another and to complete transposition is discussed. Ways by which the genetic identity of the three different forms of partial transposition with one another and with complete transposition can be proved or disproved are given. For the following reasons it is suggested that all are determined by the same gene rather than by a series of allelomorphous genes. The three forms of partial transposition are not sharply separated, i.e., there is almost perfect gradation leading up to complete transposition. Congenital morbus cordis is much commoner in partial than in complete transposition, and the anomalies of development of the thoracic and abdominal organs that occur occasionally in the complete form are much commoner in the incomplete forms. Developmental anomalies are more likely to occur with sinistral than with dextral rotation of the viscera, even when sinistral rotation is complete, they are much commoner, however when sinistral rotation is incomplete. Since many of the anomalies shorten life the gene is partially lethal.

FROM AUTHOR'S SUMMARY

ELECTIVE INSULAR (PAN-) AMYLOIDOSIS OF THE PANCREAS N GLLIFSTEDT, Beitr z path Anat u z allg Path **101** 1, 1938

In 181 autopsies, including only 3 on patients with diabetes, insular amyloid was revealed in the pancreases of about 45 per cent of the persons over 50 years of age. In younger persons such deposition of amyloid was extremely rare and never severe. The deposits of amyloid are interpreted as a peculiar morphologic expression of senility. So-called hyaline degeneration of the islets of Langerhans in diabetes mellitus is identical with insular amyloidosis and is not specific.

R J LEBOWICH

GENESIS OF CONGENITAL HYDROPS G LIEBEGOTT, Beitr z path Anat u z allg Path **101** 319, 1938

In 2 instances of congenital hydrops there was observed, in addition to the usual anatomic changes, pronounced enlargement of the strikingly numerous islets of Langerhans in the pancreas and marked increase in the width of the zona reticularis of the adrenal gland, the cells of which in the sudan preparations were loaded with neutral fat. These changes were accompanied by massive storage of glycogen in the myocardium, which was interpreted as an expression of hyperinsulinism. It is conjectured that the hyperplasia of the islets is related to a disturbance of the maternal carbohydrate metabolism, and this conjecture is to be subjected to experimental investigation.

R J LEBOWICH

HYPERTELORISM (GREIG) K BOJLEN and T BREMS, Acta path et microbiol Scandinav **15** 217, 1938

Since 1924, when Greig described the condition of ocular hypertelorism, 42 cases purporting to be cases of this anomaly have been reported. On critical analysis of the reports it is found that only 27 of the 42 cases may be taken with a fair degree of certainty to be cases of hypertelorism in the sense defined by Greig. Some of the errors in diagnosis are attributable to the difficulty of differentiating between this anomaly and other malformations that may be associated with great breadth between the eyes (congenital facial or nasal clefts, tumor formation over the root of the nose, Apert's and Crouzon's cranial dysostoses).

On the basis of these 27 rather well established cases of hypertelorism and the cases recorded in the present article, a review is given of the symptom complex of this deformity (the question of defective intelligence, the physiognomic characteristics and the combination with other deformities and diseases). As to the mental defect in particular, this is by no means a constant feature of the symptom complex, even though the combination of hypertelorism and mental defect probably occurs too often to be considered an accidental coincidence.

Familial hypertelorism is not recognized generally to occur. In this paper mention is made of 11 cases among 24 members of the same family, hypertelorism appearing through five generations as a hereditary deformity, transmitted as a dominant character. So in these cases the question of heredity is elucidated fully.

As to the causes and pathogenesis, the cases reported here furnish no evidence in support of new views. It is a question whether hypertelorism represents a primary disturbance in the development of the bones that may be accompanied by disturbances in the development of the brain, or whether it represents a primary disturbance in the development of the brain that needs not result in mental defect. The hereditary appearance of the deformity in our cases suggests that the primary factor might be looked for in an anomalous development of the prosencephalon.

FROM AUTHORS' SUMMARY

## Pathologic Chemistry and Physics

ROENTGEN RAY DIFFRACTION ANALYSIS AS APPLIED IN PNEUMONOCOINOSIS  
H C SWEANY and R KLAAS, J A M A **112** 610, 1939

Roentgen ray diffraction analysis is characterized first of all by its great specificity. Nearly all crystalline compounds give patterns each distinctly different from the others. Hence, when chemical methods are not available for identification of the type of silica present and when microscopic methods are inadequate for the study of mineral particles of the size encountered in tissue, the roentgen method gives definite information as to the identity of the crystalline substances present. Furthermore, a comparison of the patterns with regard to

the nature of the lines, i e, whether they are smooth or dotted, indicates that there is a variation in size of particles from case to case. In general, it may be concluded that the particles are 1 micron or less in diameter. Further work remains to be done on this aspect of the problem.

Another advantage of the roentgen technic described is that the tissue can be used for analysis unmodified by chemical treatment. Whenever the tissue is digested with some reagent one must make the questionable assumption that no changes take place in the mineral deposits. In the authors' work such an assumption has been unnecessary.

A further significant feature is the great sensitivity of the method. With the present technic the authors are able to detect quartz in a concentration as low as 0.2 per cent. This concentration has been found in previous work to constitute the threshold of the pathologic level. Hence, the finding of a faint line on the roentgen film corresponding to the 3.34 angstrom unit spacing of quartz appears to be a good indication of the presence of sufficient quartz to have already caused, or to be in the process of causing, specific fibrosis in the tissue. Thus the method should be of value in medicolegal work whenever difficulty is experienced in determining the presence or absence of silicosis. FROM AUTHORS' SUMMARY

BEHAVIOR OF THE HEMOGLOBIN AFTER BLOOD TRANSFUSION W. L. SIBLEY and J. S. LUNDY, Surg., Gynec. & Obst. **67** 293, 1938

In the average case in which 500 cc. of citrated blood was given by transfusion there was an increase in hemoglobin of about 1.5 Gm. in 100 cc. of blood (about 9 per cent). This took place at the end of the second day after transfusion. The addition to the hemoglobin tended to decrease to about 1 Gm. in 100 cc. by the tenth day after transfusion. An increase in the hemoglobin of 2.12 to 2.8 Gm. in 100 cc. (12.72 to 16.8 per cent) could be anticipated in cases in which the patient showed no reaction or bleeding following transfusion. The amount of increase in the hemoglobin of the recipient of 500 cc. of citrated blood was directly proportional to the value for hemoglobin before the transfusion. As a rule, there was definitely less increase (usually 50 per cent) in the hemoglobin of the recipient when a reaction to transfusion occurred than when none occurred.

FROM THE AUTHORS' SUMMARY (WARREN C. HUNTER)

DISAGGREGATION OF PROTEINS BY ENZYMES C. G. POPE, Brit. J. Exper. Path. **19** 245, 1938

A preliminary report is made on the action of fibrinolysin in producing disaggregation of the molecule of antitoxic pseudoglobulin into its protein components, each having different physical and chemical properties. Such action is not limited to fibrinolysin but appears to be a property of all proteolytic enzymes, provided they are used under the correct conditions. By taking advantage of this property of enzymes, Pope evolved a method of critical differential heat denaturation for the further purification of antitoxins. To be of use in this method the action of the enzymes must be so limited that no hydrolysis or digestion in the generally accepted sense takes place, if this occurs, differential denaturation fails, because obviously it cannot affect noncoagulable protein fragments. Based on the methods outlined, a process for large scale purification of antitoxins has been evolved and will form the subject of other papers. As a point of interest it may be stated that by the use of these methods antitoxin has been prepared experimentally which is of such purity that all the protein present can be specifically precipitated by diphtheria toxin.

FROM AUTHOR'S SUMMARY

DIFFUSE ENDOCARDIAL THICKENING IN INFANTS H. LOH, Beitr. z. path. Anat. u. z. allg. Path. **101** 253, 1938

In 3 instances of diffuse and uniform fibrosis of the endocardium of the left and right ventricles of the infant heart, errors of development were observed,



such as hypoplasia of the right ventricle After exclusion of a thrombotic and an inflammatory origin it was concluded that diffuse endocardial fibrosis represents a developmental error

R J LEBOWICH

### Microbiology and Parasitology

PASSAGE OF PNEUMOCOCCI INTO LYMPHATICS R Z SCHULZ, M F WARREN and C K DRINKER, J Exper Med **68** 251, 1938

When type III pneumococci that were virulent for rabbits were instilled into the nose or trachea of the animal, they were recovered in the lymph collected from the lymphatics draining the area involved, during a subsequent four hour period The detection of the organisms rarely failed and not infrequently was possible at the end of the first hour Practically invariably the organisms appeared in the lymphatics and subsequently in a few cases in the blood during the four hour test period Intravenous administration of antiserum two and one-half to three hours before the instillation of the organisms decreased the number of animals in which the lymph or the blood was shown to contain the organisms and the total length of time during which the organisms were recoverable in lymph from the efferent lymphatics during the test period

FROM AUTHORS' SUMMARY

PROPAGATION OF INFLUENZA VIRUS IN GUINEA PIG FETUS O C WOOLPERT and others, J Exper Med **68** 313, 1938

The PR8 strain of the virus of human influenza was found to proliferate and disseminate widely in the tissues of fetal guinea pigs, inoculated in utero After incubation periods ranging from two to six days, large quantities of the virus, free from bacteria, were recovered from lung, liver and placenta and smaller quantities from blood and brain Although the fetuses proved to be excellent for the propagation of the virus, they evinced grossly little reaction to the infection Several series of passages from fetus to fetus were accomplished, one consisted of 10 transfers and another of 16 For serial passage the virus was inoculated intracerebrally into half-grown fetuses, and the fetal lungs were harvested forty-eight hours later as a source of virus for subinoculation It is concluded that multiplication of the virus occurred in the lungs particularly, a conclusion which may be considered a significant reaffirmation of the statement that this virus shows pneumonotropic tendencies Following passage in series the virus was found, on the basis of cross immunity and cross neutralization tests, to be immunologically identical with the mouse passage virus from which it was derived Other properties also appeared to be unaltered by passage of the virus under these conditions

FROM AUTHORS' SUMMARY

CONDITIONS IN THE SKIN OF TUBERCULOUS GUINEA PIGS AS DEMONSTRATED WITH A VITAL DYE A L JOYNER and F R SABIN, J Exper Med **68** 325, 1938

The skin of tuberculous guinea pigs while it is allergic permits the spread of a vital dye, pontamine sky blue, and the drainage of the dye into the vascular system to take place much more slowly than in the normal animal The skin of moribund tuberculous guinea pigs, animals no longer allergic, permits dye to spread more rapidly than in the normal animal In the skin of guinea pigs infected with a hemolytic streptococcus the spread of dye was found to be somewhat restricted These animals were allergic The observations suggest that the dye method may disclose altered tissue conditions in the allergic state

FROM AUTHORS' SUMMARY

CELLULAR REACTIONS TO TUBERCULO-PROTEINS F R SABIN, J Exper Med **68** 837, 1938

Tuberculo-protein in solution induces formation of monocytes in animals that are normal and tubercles of epithelioid cells in animals that are tuberculous

Freshly precipitated tuberculo-proteins from the culture mediums and from the bacilli induce moderate formation of epithelioid cells in normal animals and more marked formation of such cells in the tuberculous. Insoluble forms of tuberculo-protein induce complex tuberculous tissue in normal animals. This action is enhanced in the tuberculous animals.

FROM AUTHOR'S SUMMARY

CELLULAR REACTIONS TO DEFATTED TUBERCLE BACILLI F R SABIN and A L JOYNER, *J Exper Med* **68** 853, 1938

The cellular reactions to defatted tubercle bacilli are complex and like those to heat-killed whole tubercle bacilli. The firmly bound lipid when removed from these organisms is nonacidfast, it contains a hydroxy acid which is acid-fast and a polysaccharide which is not. This hydroxy acid gives rise to foreign body giant cells, and the tissues eventually become infiltrated with eosinophils. The polysaccharides from the defatted bacilli and from the unfiltrable lipid call neutrophils from the blood stream. The reactions to the unfiltrable lipid include those to both its constituents.

FROM AUTHORS' SUMMARY

ON THE INHERITANCE OF THE AGGLUTINOGENS A, B, M AND N F E HOLFORD, *J Infect Dis* **63** 287, 1938

The adequacy of the reagents and techmics employed in the determination of the agglutinogens A, B, M and N was tested, with satisfactory results, in a random sample comprising 1,100 persons. A more critical test was represented in a study of the inheritance of the same agglutinogens in 100 families with 234 children, it revealed no exceptions to the theory of Bernstein or of Landsteiner and Levine and showed a close approximation of the observed values to the theoretic requirements. A study of 124 mothers with 277 children offered further confirmation of the adequacy of the reagents and procedures employed.

The data, showing no exceptions to the requirements of the theories of Bernstein and of Landsteiner and Levine, offer further confirmation of those theories.

FROM AUTHOR'S SUMMARY

TOMATO BUSHY STUNT VIRUS F C BAWDEN and N W PIRIE, *Brit J Exper Path* **19** 264, 1938

The isolation of a protein, probably the virus itself, from plants infected with tomato Bushy stunt virus is described. Not only does this protein differ from the normal plant proteins, but it differs more from the other purified plant viruses than these differ from one another. It is fully crystalline instead of liquid crystalline. It has a higher nucleic acid content than tobacco mosaic virus or the X virus of potato and is more stable toward  $p_H$  changes but less stable toward dehydrating agents. Its particles are not elongated, and liquid and solid preparations are isotropic. One cubic centimeter of a solution containing  $10^{-7}$  Gm produces infections when rubbed on to *Nicotiana glutinosa*, and 1 cc containing  $10^{-6}$  Gm gives a specific precipitate with antiserum. Precipitates of the rod-shaped viruses obtained with their antiserum resemble those obtained with bacterial flagellar (H) antigens, but those of Bushy stunt virus resemble those obtained with somatic (O) antigens. When irradiated with ultraviolet rays or treated with nitrous acid, the virus loses its infectivity, but it can still be crystallized and it still retains its serologic activity.

FROM AUTHORS' SUMMARY

CYTOPLASMIC INCLUSIONS IN THE ENGORGING TICK J D GREGSON, *J Path & Bact* **47** 143, 1938

Cytoplasmic inclusion bodies of the engorging gut cells of *Dermacentor Andersoni*, resembling in many ways certain living symbionts, are described with refer-

ence to their staining reactions and morphologic appearances. They are globoid, and they grow in size from ultramicroscopic dimensions to 15 microns in diameter. They are exceedingly resistant to strong salt solutions, acetic acid and fat solvents. They react feebly with all stains except Mallory's triple stain, in which they are colored crimson, bodies appearing blue by this method are thought to be old and disintegrating. Other inclusion bodies exhibiting similar appearances and staining reactions are described within the engorging tick, and an attempt is made to establish a relationship between them that might lead to understanding of their origin and fate and of their relation to the tick. These other bodies have been noted in the tick's hypodermal cells, ova, spermatozoa and phagocytic cells and in surrounding muscle fibers. To date no success has followed various attempts to culture these bodies, although in several instances forms suggestive of binary fission have been noted.

FROM AUTHOR'S SUMMARY

TISSUE AFFINITIES OF VACCINE VIRUS. C. LEVADITI, R. FASQUELLE, L. REINIE and R. SCHOEN, *Ann Inst Pasteur* **60** 142, 1938

Vaccine virus is composed of two elements, one (E) having an affinity for ectodermal tissue and the other (M) having an affinity for mesodermal tissue. In bovine dermovaccine the E elements predominate, but in rabbit neurovaccine the M factors are dominant.

Dermovaccine of bovine origin produces little or no reaction when injected intraperitoneally or intrapleurally into rabbits. Neurovaccine gives marked pleural and peritoneal reactions when injected in the ways named.

Dermotropic virus shows a strong affinity for the glandular and excretory organs (kidney and liver) but little affinity for hemopoietic tissue (bone marrow, spleen and lymph nodes) when inoculated directly into these organs. When inoculation is made into organs directly, the functional character of the organs dominates over the factor of embryonic origin, so that organs of mesodermic derivation are invaded by dermovaccine. The mesodermotropic virus attacks both groups of organs. The lymph nodes are least frequently invaded by either virus.

Different strains of dermotropic virus vary greatly in their power to produce encephalitis. Dermovaccine may become quantitatively transformed into neurovaccine by successive intracerebral inoculations into rabbits, so that the epitheliotropic property (E) of Jennerian vaccine becomes secondary to the neurotropic property (M). Nerve tissue and sometimes testicular tissue are selective for dissociation of E to M. This transformation may be produced also by inoculation into embryonic tissue or by passage through certain animal species, especially the rabbit.

This selective transformation is not reversible. If neurovaccine is inoculated intradermally into calves, no macroscopic pustules are produced. The virus can be recovered from microscopic edematous lesions and is unaltered in its neurotropic properties. Serial transfer from calf to calf results in typical pustules after about the third passage. In spite of this enhanced cutaneous virulence for the calf, the recovered virus still produces pleuroperitoneal lesions and encephalitis in the rabbit, i. e., the mesodermotropic affinities are unaltered or may even be augmented.

J. B. GUNNISON

### Immunology

TUBERCULIN PROTEIN AND POLYSACCHARIDE MOLECULES. F. B. SEIBERT and others, *J. Exper. Med.* **68** 413, 1938

Studies have been made by means of sedimentation in the ultracentrifuge and by diffusion and electrophoresis to determine the molecular weights and homogeneity of the tuberculo-protein and polysaccharide molecules as found in their natural state in unchanged filtrates from culture mediums after growth of tubercle bacilli. These results have been compared with data obtained on fractions isolated

from them or from old tuberculin by chemical procedures. By means of electrophoresis in the Tiselius apparatus it was possible to separate the protein from the polysaccharide as these two fractions occur naturally in the filtrates from the original culture mediums that have supported growth of acid-fast bacilli.

FROM AUTHORS' SUMMARY

ANTI-LYMPHOCYTIC SERUM W B CHEW and I S LAWRENCE, *J Immunol* **33** 271, 1937

Rabbits were each given twenty-one daily intravenous injections of 0.1 cc of lymphocytes. These cells had been obtained by grinding lymph nodes of guinea pigs, suspending the pulp in saline solution and filtering. A control serum was prepared by inoculating rabbits with suspensions of ground liver and kidney from mice. Both serums were absorbed repeatedly with the red cells of guinea pigs to remove the hemolytic action and then with the red cells of sheep to remove the Forssman heterophilic fraction. They were then inactivated, preserved with phenol and passed through a Seitz filter. When guinea pigs were given intracardial injections of from 0.25 to 0.35 cc of the serum, which was very toxic in higher doses, the lymphocytes dropped within a few minutes from an average of over 3,000 to less than 1,000 per cubic millimeter and remained at this level for about six hours, after which their number increased gradually. The number of neutrophils decreased similarly but only for about one hour and then rose rapidly. The control serum was not toxic and caused only a brief rise in lymphocytes. Intra-abdominal injections of the immune serum produced a similar drop in lymphocytes, except that the minimum level lasted longer, there was no drop in the neutrophils, but a marked rise. Repeated intra-abdominal injections brought about a drop in lymphocytes which continued during the entire period of treatment, while the neutrophils showed rises separated by drops. At necropsy the lymphoid tissues were hyperplastic in all animals, including the controls. The bone marrow was hyperplastic but more so in the animal receiving the anti-lymphocytic serum.

I DAVIDSOHN

FLOCCULATION OF ALCOHOLIC RED CELL EXTRACTS BY DIFFERENT TYPES OF HUMAN HETEROGENETIC HEMAGGLUTININS F SCHIFF, *J Immunol* **33** 315, 1937

Alcoholic extracts of red blood cells of the rabbit, sheep, ox and horse were flocculated only by undiluted normal human serums or by these serums diluted 1:2. Serums of persons who had been treated with horse or rabbit serums gave strong flocculation reactions with the aforementioned extracts, the serums of the latter group flocculated, in addition, extracts of red cells of cats. Serums of patients with infectious mononucleosis flocculated these extracts feebly or not at all. An alcoholic extract of guinea pig kidney (Forssman antigen) was flocculated strongly by a homologous immune serum at 37 C, it was flocculated weakly at 20 C, at the same temperature it was flocculated strongly by the serum of a patient with horse serum disease and moderately by the serum of a patient with rabbit serum disease, while the serum of a patient with infectious mononucleosis did not flocculate the extract at either temperature.

I DAVIDSOHN

IMMUNITY TO THE VIRUS OF PSITTACOSIS S P BEDSON, *Brit J Exper Path* **19** 353, 1938

Immunity to the virus of psittacosis can be produced in mice by means of a formaldehydized vaccine in which no living virus is demonstrable. This immunity lasts for at least three months. The protection obtained is not complete even against small doses of virus, since a test injection of active virus almost invariably results in infection. Multiplication of the virus, however, stops short of that

required for the production of frank disease, the infection remaining silent. This silent infection has been found to last for as long as seven months. Serial passage of virus in immunized mice through five generations and extending over a period of two hundred and ninety days produced no appreciable change in its virulence. Specific antibody can be demonstrated in low concentration in the circulation of immune mice by the neutralization test. The injection into mice of an apparently neutral serum-virus mixture results in a silent infection, which, as in the case of the actively immunized mice, may last for months. The mechanism of immunity to the virus of psittacosis is discussed, and it is suggested that it is principally a function of specific antibody.

FROM AUTHOR'S SUMMARY

STREPTOLYSINS E. W. TODD, J. Path & Bact **47** 423, 1938

Hemolytic streptococci of group A produce two distinct varieties of streptolysin—streptolysin O, which is sensitive to oxygen, and streptolysin S, which is highly soluble in serum. These are neutralized by separate antibodies, which appear to be entirely unrelated. Reduced filtrates from cultures in dextrose broth contain streptolysin O but not streptolysin S. The streptolysins which are formed by group A strains in sugar-free broth or in serum broth and the hemolytic extracts prepared by Weld's technic are all mixtures of the two lysins. All filtrates of cultures of group A hemolytic strains, including serum-streptolysin, serum-free streptolysin and Weld's hemotoxin, when inoculated into animals cause an increase in the antistreptolysin O titer of the serum but no increase in the antistreptolysin S titer, hence streptolysin O is antigenic, but streptolysin S is apparently not antigenic when separated from hemolytic streptococci. Antibodies to both lysins are formed when animals are inoculated with living cultures of group A hemolytic strains. Patients infected with hemolytic streptococci do not usually acquire high antistreptolysin S titers, although their antistreptolysin O titers may be considerably raised. The relationship between leukocidin and the two forms of streptolysin is unknown.

FROM AUTHOR'S SUMMARY

GENERAL REACTION TO INTRAVENOUS INJECTION OF MELITIN P. DURAND, Arch Inst Pasteur de Tunis **27** 193, 1938

Intravenous injection of melitin into persons without a history of possible exposure to *Brucella melitensis* produces no reaction. In persons who are or who have been infected with this organism such an injection produces a marked febrile reaction of several hours' duration. This specific shock is often a more sensitive test for brucella infection than the intradermal reaction. Intravenous and intradermal tests indicated a high incidence of this infection in Tunis, Tunisia.

FROM AUTHOR'S SUMMARY

BLOOD GROUP PROPERTIES IN SHEEP T. ANDERSEN, Ztschr f Rassenphysiol **10** 104, 1938

Of 41 rabbits immunized with human A<sub>1</sub> blood cells, 16 produced group-specific hemolysins for sheep blood. By suitable absorption tests with sheep blood it could be shown that many of these immune serums contained several qualitatively different antibodies, as shown by the reactions of the absorbed serums with various sheep bloods, one serum containing as many as five distinct hemolysins. Andersen explains these reactions by postulating the existence of different partial antigens in different sheep bloods, all of which are present in human A<sub>1</sub> blood. In several cases sheep hemolysins could be absorbed with human O blood, but immunization of rabbits with human O blood failed to elicit sheep lysins. Tests on the blood of 93 sheep with three different absorbed anti-A immune rabbit serums revealed a certain correlation between the reactions of these serums and the three group classification by isohemolysis. Sheep cell lysins obtained by immunizing rabbits with sheep blood were not absorbable by human A<sub>1</sub> blood.

A. S. WIENER

## Tumors

CARCINOMA OF THE KIDNEY IN THE LEOPARD FROG B LUCKE, *Am J Cancer* **34** 15, 1938

The leopard frog is commonly affected with adenocarcinoma of the kidney. This tumor, as in the case of mammalian neoplasms, remains localized while it is small and in its early stages, but when large it frequently forms secondary tumors in distant organs. The dissemination usually takes place by way of the blood stream. In the present paper Lucke reports 22 new examples of metastasis. His frequent observations of metastasis make the evidence for the malignancy of this tumor complete.

FROM AUTHOR'S SUMMARY

LEIOMYOMA OF THE ORAL CAVITY A P STOUT, *Am J Cancer* **34** 31, 1938

Two examples of leiomyoma are reported. One, a vascular type, developed in the base of the tongue and was probably derived from the smooth muscle of blood vessels. The other, a pedunculated tumor springing from the dorsum of the tongue, was probably a dysontogenetic circumvallate papilla. Four other reported examples of leiomyoma in the oral cavity are reviewed. It is suggested that an explanation for the apparently rare occurrence of leiomyoma in the oral cavity is to be found in the paucity of smooth muscle in that part of the body.

FROM AUTHOR'S SUMMARY

MONOCYTIC LEUKEMIA AND OTHER NEOPLASTIC DISEASES J FURTH and O B FURTH, *Am J Cancer* **34** 169, 1938

A leukemia-like disturbance, with malignant cells resembling monocytes, occurred in approximately 9 per cent of 96 mice that received intrasplenic injections of benzpyrene. This disease, hitherto not described in mice, did not occur among an equal number of control mice of the same stock but was observed on rare occasions in different untreated mice of the stocks (Rf) used in these studies. Intrasplenic injection of benzpyrene into mice increased the incidence of pulmonary tumors approximately three times. Microscopically the growth in the mice given benzpyrene was similar to that in the control mice. It appears to originate from the alveolar epithelium, often in several foci in the same animal. Its pathogenesis has not been determined, but in these experiments inhalation of the cancerogenic chemical is excluded. The incidence of myeloid leukemia was greater among the mice receiving the injections than among the controls, but further data are needed to determine a causal relationship between the injections of benzpyrene and the incidence of this disease. In each of 2 animals atypical sarcoma, possibly with its origin in muscle cells, occurred at the site of injection and led to almost complete destruction of the spleen. In the first instance the neoplasm was successfully transmitted to all of 7 mice by means of fragments introduced into the subcutaneous tissue. In 2 mice that received intrasplenic injections of cancerogenic chemicals neoplastic hemangioma of the liver and spleen occurred. Since similar neoplasms are very rare in mice and were not observed among the controls, it is possible that in these mice neoplastic hemangioma was produced by the injected chemical.

FROM AUTHORS' SUMMARY

INCREASED SUSCEPTIBILITY TO BROWN-PEARCE CARCINOMA J W MU, *Am J Cancer* **34** 407, 1938

Subcutaneous administration of an estrogenic substance (prepared from a butyl alcohol extract of the urine of pregnant women) to male albino rabbits accelerated the rate of growth of the Brown-Pearce epithelioma at sites of primary implantation and stimulated the development of metastases and also the size of the tumor growths in such foci.

FROM AUTHOR'S SUMMARY

GROWTH PROCESSES INDUCED BY ESTROGENIC HORMONES L LOEB, V SUNTZEFF and E L BURNS, *Am J Cancer* **34** 413, 1938

Among approximately 500 mice, the majority of which had received injections of various doses of estrogen over different periods of time, microscopic study of the sex organs showed in no case cancerous changes or even true precancerous proliferations. There is reason for assuming that the reactivity of the uterine epithelial structures to growth stimulation is less than that of the corresponding tissues in the vagina, cervix and mammary gland—an assumption which is in accord with the behavior of these tissues during the sexual cycle and during pregnancy. One may therefore conclude that cancerous transformation depends on, among other factors, the product of the intensity of the growth stimuli acting on a tissue and the responsiveness of the affected tissue. There are indications that in a similar manner the transformation of cylindric epithelium into transitional or squamous epithelium also depends on the product of the intensity of certain effect growth stimuli and an inherited responsiveness of these tissues to these stimuli. In a minority of the mice in these experiments certain changes were observed which represent abnormal but noncancerous growth processes, namely, penetration of the uterine glands into or through the musculature of the uterus and metaplasia of the cylindric surface epithelium and perhaps also of some glands into transitional or squamous epithelium. In a considerable number of cases, however, the squamous epithelium owed its origin to regenerative processes which led to extension of the epithelium of the cervix into the uterus. It is possible that in cystic transformation of the uterine glands, also, growth stimulation may be involved. All these changes may occur, although with much less frequency, in control mice not receiving injections of the growth-stimulating factor, becoming as a rule more frequent with advancing age. Under the influence of estrogen they are produced, on the whole, the more readily the greater the dose used and the more continuous its action.

FROM AUTHORS' SUMMARY

CYSTEINE HYDROCHLORIDE IN THE TREATMENT OF ANIMAL TUMORS J L CARR, C L CONNOR and L L GINZTON, *Am J Cancer* **34** 428, 1938

The treatment of three different rat tumors and the Brown-Pearce rabbit carcinoma with cysteine hydrochloride was practically ineffective except that direct injection of the drug into the Jensen sarcoma and an adenofibroma of rats caused complete regression and cross immunity between the two. The treatment prolonged the average life of rats bearing the Walker tumor by eleven and one-tenth days. Related substances, even those containing the SH radical (ethyl mercaptan and a compound of aldehydes and thio acids) had no effect. The experiments showed some variation in response in the various tumors treated but showed also that intravenous injections of extremely large quantities of cysteine hydrochloride, which contains the SH radical, produce little effect on a rapidly growing rabbit carcinoma.

FROM AUTHORS' SUMMARY

LYMPHOSARCOMA CELL LEUKEMIA R ISAACS, *Ann Int Med* **11** 657, 1937

Of 43 patients with lymphosarcoma, 15 entered a leukemic phase. Sternberg called it leukosarcoma, but Isaacs prefers the term "lymphosarcoma cell leukemia." The lymphosarcoma cell has a characteristic feature—a single nucleolus with a deeply hyperchromatic rim. In the immature lymphocyte or lymphoblast there are, as a rule, multiple nuclei without the rim. Supravital staining also shows characteristic features.

Such cells were found already in the aleukemic phase of lymphosarcoma, they constituted from 3 to 30 per cent of cells in the differential count. From the total white cell count of from 6,000 to 10,000 in the aleukemic phase, the cells rose in the leukemic phase to an average of 70,000, and the highest was 156,000 per cubic millimeter. The number of lymphosarcoma cells increased, too, reaching occasionally 98 per cent. There was increasing anemia, with a color index of around

1 or slightly lower. The platelets were normal at first, with a drop later in the disease. The leukemic phase was characterized by exacerbation of symptoms and by fever. The effect of roentgen therapy was not favorable. At the necropsy all the lymphoid tissues were found transformed in varying degrees into the lymphosarcoma type, and an infiltration of almost all organs and tissues was present. The leukemic phase seemed to depend on extensive infiltration of moving organs, particularly the lungs. According to Isaacs, the disease appears to be true lymphosarcoma cell leukemia and not lymphosarcoma turning into lymphatic leukemia.

I. DAVIDSOHN

RADIOSENSITIVE AND NONRADIOSENSITIVE CARCINOMA OF THE LARYNX. W. HARRIS and P. KLEMPERER, *Arch. Otolaryng.* **28**: 355, 1938.

Harris and Klempeier report on 32 cases of laryngeal carcinoma in which the only treatment was roentgen irradiation (Coutard). In every case the lesion occurred on the epiglottis or within the larynx. The roentgen therapy resulted favorably in 20 of the 32 patients, 12 failed to respond. The biopsy material was studied histologically to determine criteria for pathologic differentiation of radiosensitive and radioresistant carcinoma. The grade of cellular differentiation, the mitotic count, the anaplasia of the cells, the reaction in the stroma and the location of the carcinoma were fully considered. There were no pathologic criteria except possibly the number of mitoses which permit differentiation between radiosensitive and radioresistant laryngeal carcinoma if protracted fractional roentgen therapy is used. The results seem to contradict the belief that radiosensitivity depends on the degree of differentiation of the tumor cells.

IMMUNOLOGIC REACTIONS OF THE VIRUS OF RABBIT PAPILLOMA. J. G. KIDD, *J. Exper. Med.* **68**: 703, 725 and 737, 1938.

The evidence as a whole favors the view that the virus stimulates the formation of the virus-neutralizing and complement-binding antibodies *in vivo*, and many facts indicate that it is closely associated and in all probability identical with the antigen that reacts with immune serum to fix complement *in vitro*.

FROM AUTHOR'S SUMMARY

NORMAL AND PATHOLOGICAL DEVELOPMENTS FROM THE CELLS LINING THE GRAAFIAN FOLLICLE. W. S. GARNER, *Surg., Gynec. & Obst.* **67**: 455, 1938.

Commonly spoken of as germinal epithelium, the layer of cells lining the graafian follicle have been shown by Pedro Ramon to be connective tissue. While this readily explains the normal course of these cells to the formation of the corpus albicans, it makes difficult the explanation of the development from granulosa cells of several types of epithelium in the evolution of pathologic growths. Both of these difficulties are readily obviated if one remembers that the lining cell has the same embryonic origin as the ovum, the latter being merely a cell set apart from its fellows. Since the cells lining the graafian follicle are closely related to the totipotent ovum, it is not surprising that they may, under varying stimuli, produce a variety of types of cells. At first called germinal epithelium, the cells lining the primordial follicle are termed lutein cells after the mature follicle has ruptured, these in turn as they become hyalinized are called connective tissue cells, in the atretic cysts they are known as granulosa cells. Histologically Gardner has traced the development of granulosa cells into several types of epithelium, including the goblet type characteristic of adenocystoma, the papilloma and in a single instance almost certainly the early stages of the formation of a dermoid cyst. The observations were made on small cysts that still retained a part of the original structure so that their nature could not be questioned. Since it is reasonable to assume that the normal changes in the cells of the graafian follicle are due to the action of hormones, it may be speculated whether the abnormal changes are not due to a similar influence.

WARREN C. HUNTER



MODE OF INCEPTION AND LATERAL SPREAD OF CERTAIN SQUAMOUS CELL CARCINOMAS A BRUNSCHWIG and D TSCHETTER, Surg, Gynec & Obst **67** 715, 1938

Microscopic study of small, early squamous cell carcinomas of the skin and buccopharyngeal mucosa reveals involvement of a segment of the epithelium rather than origin from one cell or from a small nidus of cells. At the margins are zones of direct continuity between normal and abnormal epithelium where it is impossible to determine which cells are normal and which are neoplastic. Likewise, in some well established carcinomas the presence of long adjacent parallel columns of downward proliferating cells instead of a thin superficial segment affords evidence of a segmental origin of the lesion. It is recognized that besides the aforementioned methods of extension well defined carcinomas may show sharp demarcation or growth under or over the epithelial surface from which they have originated. The hypothesis of progressive cancerization as a factor in the lateral spread of such lesions is not new but has received little emphasis and has never been subjected to experimental tests.

Epidermoid carcinoma was induced in mice by repeated paintings with a 0.3 per cent solution of methylcholanthrene in benzene. By means of india ink tattooings of the skin peripheral to the induced growths it was found that the expanding carcinoma did not push the dots outward but grew over them, thus indicating progressive cancerization rather than expansive spread. Furthermore, a zone of direct continuity between neoplastic and non-neoplastic epithelium was seen where a differentiation between the two was not possible. In another group the carcinoma was bisected, and the healthy skin was approximated to the remaining half of the tumor and allowed to heal. Under these conditions the neoplasm continued to spread on the side opposite the wound but rolled up where the skin had been interrupted. These observations are interpreted to indicate that the normal skin offered resistance to carcinomatous transformation whereas that in direct continuity on the opposite side did not. It is concluded that the experiments offer support for the hypothesis that there is continued lateral spread of certain squamous cell carcinomas by progressive cancerization of normal epithelium at the immediate periphery of the carcinomatous growth, in addition to centrifugal expansion by multiplication of cells.

WARREN C HUNTER

A METASTATIC DEPOSIT OF BRONCHIAL CARCINOMA IN A HYDROCELE MISDIAGNOSED "ENDOTHELIOMA" R A WILLIS, J Path & Bact **47** 35, 1938

A case is reported in which a large malignant growth in a hydrocele sac was diagnosed as endothelioma of the tunica vaginalis but was shown by postmortem examination to be a metastasis from a small symptomless carcinoma of a bronchus. Some recently reported cases of supposed endothelioma and mesothelioma of serous membranes are critically reviewed. In none of them can this diagnosis be accepted. In regard to the confusing subject of primary celomic tumors, two guiding principles must be adhered to: 1. There are no distinctive histologic criteria of endothelioma. 2. The diagnosis of endothelioma requires that a complete post-mortem examination shall have excluded as a possible source of carcinoma each and every epithelial structure in the body.

FROM AUTHOR'S SUMMARY

### Technical

BLOOD TRANSFUSION IN AMERICA P LEVINE and E M KATZIN, J A M A **110** 1243, 1938

This survey reveals some increase in the use of the international classification for blood typing. This scientific and logical system will undoubtedly become still more popular when a greater number of medical schools teach it rather than the arbitrary, confusing and meaningless numberings.

The general adoption of statutes, similar to those of New York and Wisconsin, authorizing courts to accept the results of blood tests in paternity disputes, will also stimulate the use of the international system, since it is difficult to discuss the genetics of the blood groups in terms of any other classification.

With regard to transfusion methods, it is noteworthy that about one half of the hospitals surveyed employ two methods, the citrate and one or another of the direct methods. Fortunately the great majority of transfusionists employing the direct method use either the multiple syringe procedure or one of the simpler forms of apparatus which are manually operated, and have avoided any apparatus in which the blood flow is regulated by a ball in valve mechanism.

In this country transfusions are for the most part performed, at least in hospital wards, by interns, under the sometimes inadequate supervision of a resident or other member of the attending staff. Of course, the ideal organization for this purpose would appear to be that in which a transfusion team is employed in close association with the laboratory. However, with a rapidly changing house staff, such as is found in the great majority of hospitals, this is apparently believed to be possible only to a limited extent. Yet even under such conditions it is, in the authors' opinion, feasible and practical that this work be done under the direct control of a small number of trained workers, who instruct each new group of interns as they enter the hospital. Furthermore, the persons in charge might then form a liaison between the various hospitals, the local health department and the medical societies to regulate professional donors and to provide a center for the study of problems related to blood transfusion.

Some such cooperative action is required, since, as this survey reveals, many institutions lack adequate control of syphilis in both professional and volunteer donors. Until American hospitals have at their disposal donors from carefully regulated agencies, it seems essential for each hospital to perform a recognized test for syphilis immediately prior to the transfusion. This, however, does not in any way relieve the transfusionist of the responsibility of a careful physical examination of the donor.

Although the practice of selecting a compatible donor by a blood-grouping test of the prospective donor's cells, followed by a direct matching of the donor's cells and the patient's serum, is well established, incompatibility of the bloods still accounts for numerous avoidable accidents. It is probable that mistakes in selecting donors are attributable to poor technic in general and, in particular, to the use of grouping serums that are not sufficiently potent.

Many unfortunate accidents might easily be avoided if a cooperative organization of hospital, medical society and local health authority, such as that suggested, would undertake to teach recognized procedures for compatibility tests. The headquarters of such an organization might act as a local registry to which atypical blood could be sent for study and grouping and where transfusion accidents, so neglected at present, could be recorded and analyzed. Because of the widespread and increasing use of transfusion, these services, along with the control of the professional donor and measures to prevent transmission of disease by transfusion, are urgently necessary in the present American hospital program.

FROM AUTHORS' SUMMARY

THE BLOOD TRANSFUSION BETTERMENT ASSOCIATION OF NEW YORK CITY. BLOOD DONOR BUREAU. DE WITT STETTEN, J. A. M. A. **110** 1248 1938

It is safe to say that the work that has been done by the Blood Transfusion Betterment Association and the recognition which it has received in the city of New York will stimulate the organization of services along the same lines elsewhere. Any city of considerable size with its suburbs or satellite towns can establish a similar organization without great difficulty and with the promise that it will function as usefully as the New York Association.

FROM AUTHOR'S SUMMARY

ESTIMATION OF PLASMA AND SERUM PROTEIN B M KAGAN, J Clin Investigation **17** 369 and 373, 1938

A new method for the estimation of the total protein in serum or plasma is presented. It is based on the linear relationship which exists between the specific gravity and the protein content. The specific gravity is determined by a new falling drop method, which is easy, time saving and capable of being carried out with extremely small quantities of blood. It provides a measure of the protein content with an accuracy which exceeds clinical requirements, it is about twice as accurate as the refractometric method. FROM AUTHOR'S SUMMARY

A SILVER IMPREGNATION METHOD FOR RETICULUM G DE OLIVEIRA, Virchows Arch f path Anat **298** 523, 1936

De Oliveira describes a silver impregnation method for which he claims the following advantages. It is rapid, it can be used and is best used for paraffin sections, it differentiates sharply between reticulum and collagen fibrils, it stains the finest intracellular reticulum fibrils, thus permitting study of the relationship of the cells to the fibrils. For the details of the method the reader is referred to the original article.

O T SCHULTZ

# Society Transactions

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## NEW YORK PATHOLOGICAL SOCIETY

ALFRED PLAUT, *President*

*Anniversary Meeting, Jan 26, 1939*

ROBERT A. MOORE, *Secretary*

### THE NASAL REACTION OF THE FERRET TO INFECTION WITH THE VIRUS OF EPIDEMIC INFLUENZA THOMAS FRANCIS JR (by invitation)

On intranasal inoculation of ferrets with the virus of epidemic influenza (PR8 strain) an orderly sequence of events occurs, involving the nasal mucous membrane. After twenty-four hours a moderate catarrhal reaction occurs, and after forty-eight hours almost complete destruction of the respiratory epithelium is seen. The olfactory epithelium is undamaged. Associated with the acute necrosis of the respiratory epithelium is an accumulation of exudate in the air passages and an acute inflammatory reaction in the submucosa.

In four to six days repair begins with the development of a transitional type of epithelium at the previous site of the respiratory epithelium. This gradually thickens and forms a true squamous epithelium, and by the twelfth to the fifteenth day the ciliated columnar cells are returning. In the fourth week a comparatively normal respiratory mucosa is seen, but some fibrosis of the submucosa remains. Occasional rests of transitional or squamous epithelium are interspersed through apparently normal areas.

During the period of repair, when the transitional and squamous epithelium is in prominence, reinoculation of the virus produces no reaction. The epithelium is resistant not only to virus but to physicochemical stimuli which normally destroy the entire mucous membrane of the nose. Thus there is observed an altered epithelium which maintains a refractory state or a state of immunity which bears no relation to ordinary immunologic concepts.

The state of complete refractoriness is temporary and gives way to a state of susceptibility to chemical injury. This return to susceptibility is associated with the reappearance of normal ciliated columnar cells. Nevertheless, one infection conditions the mucous membrane so that with subsequent infections the repair process is found markedly accelerated.

#### DISCUSSION

A. N. ROSEN: What is the character of the exudate?

THOMAS FRANCIS JR: In the acute stage the exudate in the nose and nasal passages is essentially polymorphonuclear. Of course, there is sloughing of the epithelium along with it. In the submucosa the inflammatory reaction in the acute phase is usually predominantly polymorphonuclear, but in the animals which have received repeated inoculations and then show reaction to a subsequent inoculation the reaction in the submucosa is predominantly mononuclear.

ROBERT A. MOORE: Is it possible in ferrets to confer passive immunity so that there will be no clinical disease? If this should be possible, I wonder what would be the anatomic change in the mucous membrane if such an animal were inoculated intranasally with active virus.

THOMAS FRANCIS JR: On occasion, if one is lucky, one may have an animal which appears to become immune by that procedure. On the other hand, the majority of animals have to be given relatively large amounts of serum, and even

then one may observe a certain amount of nasal reaction. The same thing is true in vaccinated animals. One may vaccinate them and induce production of the antibody and yet not have full protection from the nasal reaction, but those are studies which are not really completed.

IRVING GRAEF. Apropos of the remarks about the apparent immunity of the reparative respiratory epithelium, I am wondering whether or not during the earliest period of sloughing induced by zinc sulfate the virus in the nose may penetrate the mucosa and perhaps invade the olfactory nerves more readily. Is there any evidence of invasion via the mucosa in the first twenty-four to forty-eight hours?

THOMAS FRANCIS JR. One has no way of detecting that from the point of view of the olfactory nerve with the PR8 strain of virus. Stuart-Harris and I tried in certain experiments, which are still not complete, the reverse procedure of instilling zinc sulfate into the nose, observing the damage and watching for the stage of repair to find out whether we could get resistance to develop. It appeared we were not getting complete immunity but a modified disease. There is a factor which we cannot control entirely. While we attempt to keep the zinc sulfate from getting into the lungs, we cannot prevent the possibility of the virus getting into the lung, and since it is adapted to the lung, one always has that as another source of infection. When one sees the type of damage which occurs with the virus alone in the acute stage, or with zinc sulfate alone in the acute stage, one thinks that the entire structure would be more susceptible to any type of infection. On the other hand, after five or six days it seems much more resistant to any type of infection.

MILTON HELPERN. Does the repair after the application of zinc sulfate occur more rapidly in an animal which has recovered from a previous inoculation of virus than in an uninfected animal?

THOMAS FRANCIS JR. I cannot answer that. We had too few animals treated in that way to decide, but we had a distinct feeling with zinc sulfate that there were more islands of undamaged tissue left than with the virus. Whether that was simply due to chance or not I do not know.

MILTON HELPERN. I wonder if the difference in reaction to zinc sulfate as shown by an uninfected animal and an animal during the stage of repair is not due to the fact that columnar epithelium is less resistant than stratified epithelium to a corrosive.

THOMAS FRANCIS JR. We appreciate that, and we also think it was probably a factor in resistance to the virus. I do not believe that this is simply a mechanical barrier. I think it is a question of the selective action of the virus or of a chemical on certain types of cells. In the repair process, which was quite striking around the eighth to the tenth day, when we had the most striking stratified epithelium, we got resistance not only in the respiratory area but also in the olfactory area, and I do not know how to explain that unless the virus had done something to the olfactory cells but not anything to cause great damage. When on the fifteenth day we added the zinc sulfate, the first cells to be destroyed were the ciliated columnar cells.

N. CHANDLER FOOT. Have you tried secondary infection with pyogenic cocci to see whether the epithelium which becomes resistant to the virus has become more susceptible to the pyogenic cocci which are observed in connection with the usual secondary infections of influenza?

THOMAS FRANCIS JR. We have not done that. We have had this experience. We had animals which were carriers of *Streptococcus*, and we inoculated them with the virus, in those animals disease and death occurred, much more rapidly with mediastinitis, pericarditis, pleurisy with effusion and septicemia. We had in the present group a certain number of animals which had a chronic bacterial infection superimposed on the virus infection, and in these animals the undifferentiated epithelium was apt to persist much longer, and the ciliated epithelium was slower in returning. In one, particularly, the whole side of the nose was in what might

be thought of as the twelfth-day stage of repair, whereas the other side was normal, and in that animal there was distinct pus in the antrum

A B SABIN How much of the respiratory epithelium is damaged by the virus?

THOMAS FRANCIS JR In the respiratory epithelium we rarely see anything remaining Occasionally we see a small island, but the surprising thing is how complete the damage is, particularly when one thinks of the structure of the anterior turbinate bone That is one of the differences between the virus and the zinc sulfate There is always much more complete damage with the virus than with the zinc sulfate, so that one can say that 99 per cent of the respiratory epithelium was destroyed by the virus, the destruction was almost complete

A B SABIN I ask that because these studies have a definite bearing on the anatomic basis of tissue immunity If this virus destroys almost all the susceptible cells, it does not share this property with many others Most other viruses usually leave large portions of susceptible tissue anatomically unaffected If immunity were limited to the cells which had been attacked and had then undergone an anatomic change, there would be no immunity in the uninvolved tissues Yet it is known that a whole organ or system may become immune when only a few of its cells have been directly affected by the virus, under conditions in which humoral antibodies play little or no role One is led to assume, therefore, that in addition to the recognizable anatomic changes, certain other, more subtle changes must occur in all the susceptible tissues to account for their acquired resistance to infection with a specific virus That holds perhaps not only for infectious agents but also for physicochemical agents I might say from personal experience with zinc sulfate instilled intranasally in monkeys that while there is early injury to some of the cells of the olfactory mucosa, large portions of it show no sign of damage, and within a week all of it may appear normal, yet for a period of several months that mucosa remains resistant to infection with the virus of poliomyelitis, indicating that resistance cannot be attributed to the disappearance of, or to the anatomic change in, the susceptible cells

THOMAS FRANCIS JR I think that is true The fact is that following inoculation the PR8 strain destroys primarily the respiratory epithelium and leaves the olfactory epithelium untouched, and yet after ten to twelve days the zinc sulfate, which would normally wipe out all the epithelium, does not damage it This is in the absence of any change in the olfactory epithelium In the lung a similar state of affairs occurs We did not get complete involvement of the entire lung, and yet the animals became resistant, and that is why we had the feeling that the tissue changes would not explain the entire picture of immunity in these instances Up to a certain point we thought they would account for immunity in the absence of any serologic and immunologic reaction in the typical sense, but after that we would not rely on them

So far as the observations on the monkey with the zinc sulfate, those changes are perhaps not quite so marked as in the present instances, because with ionization there is a pretty severe injury, and furthermore, when we used simple instillation we used it for as long as fifteen minutes, so it was not merely an application to the area Instead of the coagulation type of necrosis which one sees following local application we saw a rather destructive wiping off of the epithelium

CARCINOMA IN FROGS ITS ETIOLOGIC RELATION TO A VIRUS AND ITS HABITS OF GROWTH IN VIVO AND IN VITRO (ILLUSTRATED BY A MOTION PICTURE)  
BALDUIN LUCKE (by invitation)

The leopard frog is commonly affected with an adenocarcinoma of the kidney which, like similar tumors in man and other mammals, invades and destroys the adjacent tissue of the organ in which it grows Metastasis is commonly observed with tumors which have attained large size, dissemination usually takes place by way of the blood stream These frequent metastases make the evidence for the

malignancy of this tumor complete, further, they throw doubt on the opinion, so often encountered in the literature, that tumors of cold-blooded vertebrates have little tendency to metastasize. Indeed, these studies and others in progress support the view that no matter in what species of animals tumors of the same kind occur they are much alike in behavior as well as in structure.

The carcinoma of the frog is a particularly interesting tumor as its nuclei commonly contain large acidophilic inclusions such as suggest activity of a virus. The results of transmission experiments make it very probable that this carcinoma is, in fact, induced by a virus. When the growth is inoculated as living fragments or a cell suspension into the lymph sacs, the cranial cavity or the abdomen, no significant local growth results, and the implanted material is gradually resorbed. However, in approximately 20 per cent of the frogs surviving inoculation for more than six months, tumors develop in the kidneys which are like the spontaneous neoplasms. The incidence far exceeds that in the controls.

Desiccated and glycerinated tumor injected into the abdomen gives the same results as inoculated living tumor, in somewhat over 20 per cent of animals surviving more than six months tumors of the kidneys occur.

In alien species of frogs, no tumors are produced by inoculation, either with living or with desiccated tumor.

These experiments indicate the probability that the carcinoma of the leopard frog is caused by an inclusion forming, organ-specific virus.

Thus in an amphibian a virus is seen to be capable of producing a malignant tumor. Since it is known that viruses produce certain tumors of birds and mammals, the conclusion follows that viruses may be a frequent cause of tumors throughout the animal kingdom.

Examination of nearly 1,000 control frogs gave very different results from those of the experimental series. During the first three months period after the inception of the corresponding experiments, slightly over 2 per cent had renal tumors. This incidence rose slightly to 6 per cent in the second three month period, and to 67 per cent in frogs surviving for more than six months. While this rise is far below the striking increase in the experimental groups, it may have considerable significance. There exists a real possibility that the neoplastic disease is transmissible from frog to frog. In captivity frogs are of necessity maintained under more crowded conditions than obtain in their natural environment, and confinement in the laboratory would favor not only direct contact but also indirect transference of various agents.

The characteristics of cancer growth have hitherto been studied chiefly by histologic methods, i. e., in material that has been fixed, sectioned and stained. The recent development of slit lamp microscopy makes it possible now to observe the habit of growth of living tumors. Bits of these tumors are implanted in the anterior chamber of the eye, where they soon establish themselves and where their rate of growth may be measured. The form of the tumor as well as the arrangement of the constituent cells may be observed through the cornea by means of the slit lamp microscope. Observation of many such transplanted fragments of carcinoma has led to the conclusion that the form which the growing tumor assumes depends on its immediate physical environment. Where the tumor grows out in the midst of the aqueous humor, not in contact with cornea or iris, there the habit of growth is characteristically tubular or papillary, the projections being hollow and cystic in some instances, solid masses of cells in others. If, however, the tumor grows in contact with an even surface, such as the cornea or the iris, then the form of growth is entirely different, broad membranes are formed which extend along and cover the cornea or the iris, such growths show no sign of tubule formation or, at most, abortive tubules, appearing late in the course of growth.

Study of tumors by the method of tissue culture has yielded much information concerning the differences between malignant cells and normal adult cells of the same type.

In the present experiments 32 frog tumors have been cultured by the roller tube technic of Gey and Lewis as well as by the ordinary hanging drop method.

Under these conditions budlike projections promptly grow from the tumor explant into the solid medium, where they form structures resembling tubules except for absence of lumen. The tubules are at first contained within basement membranes, but later the proliferating epithelial cells break through the basement membrane and spread out as thin fans of polyhedral epithelial cells. These fuse with other outgrowths of similar character until the explant is entirely surrounded by a thin, flat layer of epithelium which shows no trace of differentiation into tubules or acini. The cells of the frog carcinoma are distinctly larger than those of most mammalian tumors, making the present material especially suitable for cytologic study.

In addition to direct observation, two cultures of the tumor were studied by cinematograph. This method not only affords a permanent record, but makes clearer the manner of outgrowth, the character of locomotion of the individual cells, as well as intracellular changes which occur so slowly as otherwise to escape detection.

#### DISCUSSION

JACOB FURTH. Dr Lucke interpreted the increase in the percentage of tumors with increase in time of observation among the control frogs as due probably to spontaneous transmission. In this case the addition of tumor desiccate to the water might increase the incidence of tumors. Is it feasible to do the reverse—to raise frogs in sterilized water in attempts to obtain a tumor-free stock? To return to the first comment. Is it not possible that this increase in the number of tumors is due to increasing age of the frogs, and not to spontaneous transmission? This is a fundamental question. Thousands of chickens carrying filterable tumors have been studied in many laboratories, but I do not know of a single instance of transmission of chicken tumor by spontaneous means.

BALDUIN LUCKL. It is entirely possible to do what you suggest, but practical difficulties are in the way. As one buys frogs from dealers, one gets frogs of all ages, in some twenty-odd thousand frogs which we have examined in our laboratory (we have divided them into groups of thousands) the incidence of spontaneous tumor is pretty steadily around 2 per cent in the different groups. I do not think that age has anything to do with increase in incidence among controls. I think it means that in some way the tumor is being transmitted. The fact that it does not occur in chickens simply means that it does not occur in chickens, it does not mean anything so far as frogs are concerned. Frogs suffer from all kinds of parasitic infection, and it is conceivable, though I do not offer this very seriously, that the virus is transmitted through parasites. What we are doing is keeping a large number of frogs, some of them having tumors, under as natural conditions as is possible, and these frogs are being kept together for as long a time as seems suitable. From time to time a group is examined, and the incidence of tumor is recorded. These experiments are as yet inconclusive.

IRVING GRAEF. Have you any observations on the effect of the desiccate or implants on tadpoles?

BALDUIN LUCKL. We have tried to implant desiccate as well as living material into tadpoles but have invariably failed to obtain tumors.

THOMAS J. FRANCIS JR. May I ask about the old tradition that if a toad urinates on a man's hand he gets a wart? Do any of the tumor cells appear in the urine of these frogs, and, if so, would that be a means of tumor dissemination?

BALDUIN LUCKL. Tumor cells do appear in the urine. I have repeatedly punctured the bladder of a frog that had a tumor of the kidney and have found unquestionable tumor cells in the urine. Some of these are pretty badly broken up, but they are still cells of a tumor, and it is conceivable that through them a virus may be transmitted.

AMOUR F. LIBER. Is there any evidence of hereditary transmission?

BALDUIN LUCKE. To raise frogs is a difficult thing. It is easy to bring tadpoles to the frog stage, but from there on there is a very high mortality.



ALFRED PLAUT, *President*

*Regular Monthly Meeting, March 23, 1939*

ROBERT A. MOORE, *Secretary*

MULTIPLE PLASMOMA OF THE ILEUM AND COLON CHESTER R. BROWN and AMOUR F. LIBER

A colored man aged 57 was admitted to Lincoln Hospital on Nov. 27, 1938, complaining of "itching and leaking of purulent material from the rectum," said to have been present for from ten to fourteen years. The patient had lost 75 pounds (34 Kg.) in weight in the past three years. He said that there was a "sore on the penis" at one time, and that he received treatment for syphilis twenty years ago.

The man was emaciated and chronically ill, there was a blood-tinged purulent rectal discharge. Numerous polypoid firm masses were felt encircling the rectum about 3 inches (7.6 cm.) above the anus. The Frei test on the right arm showed a reddened wheal 2 cm. in diameter. The left arm (control) was negative. The Wassermann reaction was anticomplementary. The blood showed no significant changes. Pulmonary signs developed, and the patient died on Dec. 4, 1938. The clinical diagnosis was carcinoma of the rectum and bronchopneumonia.

*Postmortem Examination*—Attached by broad bases to the external surface of the ileum throughout its entire course were numerous masses of yellow-white tissue, varying between 3 and 6 cm. in length and 2 and 4 cm. in breadth. The masses were considerably elevated above the serosal surface of the intestine and were quite firm to palpation. On section these were composed of firm, yellowish white tissue, of uniform consistency throughout, encircling about two thirds of the circumference of the ileum in localized areas. One of these nodules almost completely closed the lumen. Here the mucosa showed some superficial ulceration. The other masses did not encroach on the intestinal lumen.

The omentum and mesentery were adherent over a localized area of the hepatic flexure of the colon approximately 10 cm. in length. After removing the adhesions, a small localized abscess cavity was revealed, the base of which was formed by the exterior of the intestinal wall, in its center a necrotic opening could be followed down to the mucosa. Evidently these changes represented a localized chronic perforating lesion of the hepatic flexure. Section through this mass revealed dense yellow-white tissue involving all the intestinal coats and extending considerably above the serosal surface to form a polypoid mass with a sessile base.

The perirectal tissues showed infiltration of similar type, with marked induration. The mucosa was considerably elevated and firm, but showed no ulceration.

The mesenteric and portal lymph nodes were enlarged and of uniform soft consistency throughout.

Microscopic examination revealed the intestinal masses and lymph nodes to consist of cell infiltrations of uniform type throughout. These were solid sheets of plasma cells (about 80 per cent) and lymphocytes with varying degrees of fibroblastic induration. There were occasional macrophages. Amitotic division was frequent in the plasma cells. Polymorphonuclear cells were absent except in a small area localized to the perforation in the hepatic flexure. Reticulum cells were minimal. These collections infiltrated all the intestinal coats and the epiploon to form dense neoplastic-like masses. There was invasion of blood vessels by similar cell masses. No evident origin or localization could be noted in the lymphoid follicles of the mucosa, which were not enlarged.

The mesenteric fat was heavily infiltrated with similar cells, as were all the lymph nodes. The latter showed complete disorganization of the normal architecture. The sinuses and pulp formed solid cell masses. The capsule was invaded in many areas. Numerous periportal areas in the liver showed diffuse infiltrations of plasma cells and lymphocytes.

In the intestinal and lymphatic infiltrations pleomorphism and giant cells were absent. Reticulum cell proliferation was minimal. The invasions of the lymph nodes and of the blood vessels and the periportal accumulations suggested metastatic lesions. The changes in no way resembled the histologic picture usually ascribed to the so-called benign intestinal granuloma or regional ileitis. Stains for spirochetes and tubercle bacilli were negative. We have found no case in which a condition similar to this was described as a visceral form of syphilis. The histologic observations do not resemble those of granuloma venereum.

Four cases somewhat similar to this one are reported in the literature.

Case 1 (Vasiliu, T., and Popa, R. *Compt rend Soc de biol* **98** 738, 1928) Multiple ulcerated and nodular tumors were seen in the mucosa of the stomach and large and small intestine. The lymph nodes and the entire mesentery were invaded. Clumps of nodes compressed the ileum. Histologically, all the tumors were made up of plasma cells. The bone marrow was not examined.

Case 2 (Vallone, D. *Ann ital di chi* **9** 20, 1930) A 24 year old man was operated on because of chronic intestinal obstruction. The Wassermann test was negative. Fifteen centimeters of ileum was resected. The lumen was obliterated by a tumor composed of plasma cells and lymphocytes. The marrow was not examined.

Case 3 (North. A Case of Plasmocytoma of the Small Intestine, cited by Vallone) A woman aged 47 was operated on for intestinal obstruction. Thirty centimeters of ileum was resected. Sections showed marked infiltration of all the intestinal layers by plasma cells. The lesions were considered neoplastic.

Case 4 (Razzaboni. Di una rara lesione della parete intestinale infiltrata plasma-cellulare, cited by Vallone) There were multiple ulcerated tumors in the terminal part of the small intestine, the colon and the appendix, composed almost exclusively of plasma cells.

The origin of the plasma cell is highly controversial. Plasma cell tumors have been found in the nose and throat, lacrimal glands, conjunctiva, respiratory tract, skin, genitourinary tract and lymph nodes, without involvement of the bone marrow. Some are of malignant type (Masson, Jackson and Parker).

Although the marrow was not examined, we do not believe that ours is a case of medullary myeloma. In the latter disease the history and course are dissimilar. Metastases from medullary myeloma consist of pure plasma cells. We believe this to be a case of neoplastic multicentric plasmoma which may represent a variety of lymphosarcoma.

#### DISCUSSION

ALFRED PLAUT. There is a not very small group of intestinal lesions, more in the small than in the large bowel, which seem to be between tumor and granuloma. My colleagues and I have had 2 cases lately in our material, and I wonder whether the fact that the plasma cells predominate is something to differentiate Dr. Brown's case from others. In inflammatory lesions one does see sometimes a preponderance of plasma cells without these lesions being in other respects essentially different.

AMOUR F. LIBER. There is one feature of this case which would be difficult to explain on the hypothesis of chronic inflammatory granulomatosis, that is the presence of large collections of plasma cells in the lumens of blood vessels, in the outer coats of the affected parts of the intestine and also in the liver, where small blood vessels were packed with these cells. The cells in the lumens of the blood vessels are not part of a thrombus. They are not enmeshed in fibrin or platelets, and I am certain if one saw epithelial cells or cells which one might be inclined to attribute to a sarcoma in a corresponding situation one would not hesitate to speak of metastases going on by a vascular route.

POST-TRAUMATIC (BUT NOT POSTFRACTURAL) RAREFACTION OF LONG BONES  
HENRY L. JAFFE

It has been established on a roentgenographic basis that a blow, bump or twist to an articular or polyarticular region may be followed by pronounced rarefaction of the bones in the area in question. The instigating trauma need not have induced a fracture, and the rarefaction of bone may evolve even if the part continues to be used. This rarefaction is merely one element, however, in the complex of changes that evolve in the affected part. This complex includes rapid wasting of the local muscles and subcutaneous tissues and changes in the skin, which becomes cyanotically mottled, atrophic and scaly. In addition, the articular capsules of the part may shrivel so that articular function becomes considerably limited.

The post-traumatic syndrome is most familiar as localized in the hand or foot. Post-traumatic collapse of a vertebral body is undoubtedly another expression of it. However, material permitting adequate exploration of the pathologic-anatomic changes in this condition in these various localizations seems not to have become available.

The case being presented should contribute to knowledge of the pathologic nature of the condition in general. It also seems worth reporting in its own right as representing a rare localization of the disorder. Indeed, it hardly seems to be known at all that after trauma to a large joint region without fracture long bones, too, may undergo rarefaction. I have found in the literature reports of only 4 cases even roughly similar to the case being demonstrated. Anatomic material was not available in any of these, and in all the lesion seems to have been misdiagnosed clinically. In the case being demonstrated here, anatomic material did become available. This happened because the limb had been amputated through the upper third of the thigh on the mistaken assumption that a malignant tumor was present in the femur.

The subject, a man of 30, had injured his right knee in jumping off a wagon. He was unable to continue his work that day. Subsequently, he resumed work but had some difficulty because of painfulness and limitation of motion of the knee. Nine weeks after the injury there was a flexion deformity of the region. The latter was also tender to touch. Otherwise, the general health of the patient was good.

Roentgenograms taken about ten weeks after the injury showed profound and extensive modification of the lower half of the femur. There was complete obliteration of the spongy architectural pattern of the condyles. The condylar outlines showed notches in some places and in others were so vague that they could hardly be traced. Proximally to the condyles the rarefaction was still very pronounced. The patella, too, showed rarefaction, with irregularity and obscuration of its outline. In general, the tibia and fibula for some distance below their upper ends were also modified in this way. As noted, the changes in the femur were misinterpreted as reflecting the presence of a malignant tumor in that bone, and this is understandable in view of the ambiguous roentgenographic appearances. I myself have shown the roentgen pictures to several competent roentgenologists, giving them the pertinent facts about the case. All of them stated without equivocation that there was a malignant tumor in the femur. I mention this to show how natural the original erroneous interpretation was under the circumstances.

In the microscopic sections the bone rarefaction was also manifested in porosity of the compacta and meagerness of the trabeculae of the spongiosa. In the cortex large resorption spaces were found, filled with a loose fibrofatty connective tissue bearing numerous engorged blood vessels. The cortex also presented evidence that lively reconstruction had been going on in it. What is interesting, too, is the absence of osteophyte-like new bone deposition by the periosteum. In the spongiosa proper the intertrabecular marrow was fatty, somewhat abnormally fibrillar and slightly edematous. The original osseous trabeculae had been largely resorbed, but such trabecular fragments as had persisted also showed evidence of

reconstruction and regeneration. There was no evidence that these various changes had been preceded by aseptic necrosis.

Some years ago Dr. William Boyd sent me tissue and copies of roentgenograms in this very unusual case and gave me his permission to use this material. For further details concerning this case, for the light it sheds on the general pathologic picture of post-traumatic rarefaction of bones of the hand and foot and of vertebral bodies and for a discussion of the possible pathogenic mechanisms, the reader is referred to an article by me published this year in *Radiology*.

#### SPONTANEOUS TUMORS OF RABBITS AND THEIR TRANSPLANTATION IN THE SAME AND IN ALIEN SPECIES. HARRY S. N. GREENE (by invitation)

During the past five years, 152 spontaneous tumors have been found and studied in a colony of rabbits which averages a population of approximately 800 adults. Mammary and uterine tumors have occurred in the largest numbers, and their frequent occurrence offers an unusual opportunity for pathologic investigation.

The uterine tumors and the acinar type of mammary growths arise without observable antecedent abnormal tissue changes. On the other hand, the papillary type of tumor of the breast originates as an apparently unrelated pathologic state and progresses through noninvasive neoplasia to cancer. In such cases repeated examination of mammary tissue shows the presence of a continuous disease process in which cystic disease, benign neoplasia and invasion occur as succeeding events in the breast.

Profound endocrinologic changes distinguish animals bearing the tumors and are present from the earliest stages of tumor development. Histologically, the changes are identical with those observed in animals subjected to long-continued treatment with estrone (theelin).

The tumors have been serially transferred through many generations of rabbits and have been successfully transplanted to the anterior chambers of alien species including the guinea pig, goat, sheep and hog. At autopsy, however, none of these animals showed endocrinologic changes similar to those observed in the animals bearing spontaneous tumors. It is suggested therefore, that the endocrine changes are associated with the initiation of neoplasia and that the spontaneous tumors represent a natural analogue to the experimental induction of neoplasia with estrogenic substances.

#### DISCUSSION

AMOUR F. LIBER: The demonstration of the endocrine changes seems to be of particular significance in the rabbit if, as I believe, there is no evidence of a hereditary tendency to tumors in that species, as there is in mice. The demonstration by Lacassagne of the effect of estrogen in producing tumors of the breast was made in the males of tumor-bearing hereditary stocks, so that the demonstration of a corresponding effect in stocks presumably not bearing a hereditary factor for tumor production would be particularly important. I should like to ask whether the neuroma demonstrated was in the central or in the peripheral nervous system.

HARRY S. N. GREENE: In the peripheral nervous system.

AMOUR F. LIBER: It is striking that no tumors of the central nervous system are found in rabbits. Outside of those observed in human beings and a very small number reported in cats, tumors of the nervous system are almost unknown.

HARRY S. N. GREENE: I have made autopsies on 4,000 to 6,000 rabbits and have never seen a tumor of the stomach or of the central nervous system. Mammary tumors of the papillary type occur exclusively in Belgian hares and in Belgian-English hybrids of a single family group. The acinar type, on the other hand, occurs solely in a branch of the English breed and in hybrids derived from it. The uterine tumors are more widespread throughout the animal population but tend to occur in certain family groups. They are found with the greatest frequency in lines in which the incidence of toxemia of pregnancy is highest, and it is of interest that all animals bearing uterine tumors have recovered from one or more attacks of this disorder.

J VICTOR Is there any relationship between the sexual history of these animals and the occurrence of tumors? I wonder whether Dr Greene tried the transplantation of uterine tumors to the uterus, and if so, was he successful? Another question is whether normal tissues may be transplanted into the eyes of alien species with any degree of success

HARRY S N GREENE I have not tried to transplant normal tissues to foreign species. Homologous transplants of normal tissue grow well for a period of time and offer a convenient means for studying the possibility of a virus relationship. Fragments of normal endometrium soaked in a filtrate of uterine tumor tissue have been transferred to the anterior chamber and, while growth occurred, histologic examination at different periods showed no evidence of neoplasia.

There is a definite relation between the sexual history and the occurrence of the tumors. The series of rabbits in which tumors of the breast occurred showed reproductive abnormalities, including sterility or reduced fertility, with diminished size of litter, increased number of deadborn young and poor maternal care, for at least six months before the appearance of the mammary lesions. These abnormalities occurred with increasing frequency as the disorder progressed, but the animals were capable of becoming pregnant and bearing litters up to the time of death. Animals bearing uterine tumors are usually infertile, but a similar reduction in reproductive index occurs during the six month period preceding clinical detection of the growths. The antecedent breeding history of animals bearing both types of tumor is also distinguished by frequent resorption of fetuses.

J VICTOR Have you made any studies on the secretion of estrogenic substances?

HARRY S N GREENE We should like to do that, but we have been hindered by lack of funds and time.

ROBERT A MOORE As I understand it, a tumor of the rabbit transplanted into the eye of a rabbit gives metastases. Are there metastases when a tumor of the rabbit is transplanted into the eye of an alien species? Do you have any information concerning the source of the stroma in the alien species after several passages? Is there any way of determining whether the stroma is still rabbit stroma, or guinea pig stroma?

HARRY S N GREENE I have been anxious to get the tumors growing in as many guinea pigs as possible and as a routine have killed animals for transfer purposes shortly after growth became evident. As a result, there has been no case in which a tumor has grown in a guinea pig for a period of time equivalent to that required before metastasis occurs in rabbits. Guinea pigs now living have borne the transplanted tumors for five months and are being held to determine whether or not metastasis will occur.

I have been interested in finding out whether the stroma of the transplanted tumor in these cases was guinea pig or rabbit tissue but as yet have not got to the point of actual investigation. The point can be determined without serologic tests. The Shope myxoma is specific for rabbit connective tissue, and if a tumor in a guinea pig's eye can be infected with this virus, the identity of the stroma as rabbit tissue will be established.

B M FRIED In the early part of this century it was demonstrated by many observers that in a considerable number of cases an animal in which a transplanted tumor had developed and then receded was quite refractory to a second inoculation. The doctrine of resistance (or active immunity) to malignant disease was, however, vigorously combated and denied until Besredka came out with his conception of "local" immunity against some malignant tumors. Besredka found that the well known epithelioma of Brown and Pearce, which is almost invariably fatal when inoculated into the testicle of the rabbit, is harmless to the rodent when introduced intracutaneously. What is more, an animal in which the malignant tumor has been absorbed acquires a lasting immunity to subsequent inoculations with the carcinoma irrespective of the organ or the structure utilized. Dr Greene has just demonstrated his interesting experiments with the spontaneous cancers

which he often found in aged rabbits. He succeeded in transplanting these tumors to homologous and heterologous animals. He noticed, too, that the transplanted tumors receded after having reached considerable dimensions. I wonder whether he has attempted to ascertain whether these animals with healed tumors became immune (or resistant) to subsequent inoculations with the same malignant neoplasm.

HARRY S. N. GREENE. Simultaneous inoculation of both eyes or of one eye and a testicle gives rise to growth in both locations. If the second inoculation is delayed for thirty-five days, growth still occurs, but if the second inoculation is delayed for one hundred and thirty-five days, reinoculation is unsuccessful. It is assumed that a refractory phase develops in response to the continued presence of growing neoplastic cells. It seemed possible that the long delay of metastasis in animals bearing spontaneous tumors, in spite of the presence of neoplastic cells in the blood stream from an early period of tumor development, might be related to a similar phase. To test this possibility, tumor material derived from one of the serial eye generations was transferred to the anterior chambers of animals bearing spontaneous uterine growths of the same nature. The success of transplantation was apparently directly related to the age and size of the spontaneous growths. "Takes" occurred and subsequent growth was rapid in all instances in which the spontaneous tumor was old and large. On the other hand, complete failure or extremely slow-growing nodules which were no more than doubled in size after six months of growth followed transfer to animals with small, early uterine tumors. Histologically, the small, slow-growing nodules were characterized by an abundance of stroma and a well differentiated epithelial structure, in direct contrast to the appearance of transplants in the eyes of normal animals. It is conceivable, therefore, that a refractory phase may arise in response to the presence of the primary growth in spontaneous cases and possibly account for the long delay of metastasis.

#### SARCOMA OF THE TRACHEA. TOBIAS WEINBERG (by invitation)

Two cases of sarcoma of the trachea are reported, increasing the total of reported cases to 34. Both occurred in men, aged 50 and 34, respectively. The former gave a clinical history covering five years, and the latter one covering fourteen months. The first patient's lesion was diagnosed at biopsy as "suggestive of mixed tumor of the salivary gland type." The postmortem specimen showed the characteristics of myxosarcoma. Reexamination of all the biopsy specimens showed gradual transition into that of the postmortem picture, and because of this the possibility was considered of a unilateral development of a mixed tumor into a myxosarcoma. The second patient's tumor was typical spindle cell sarcoma. The tumors in both patients were situated both endotracheally and peritracheally, the myxosarcoma destroying the cartilages in its path.

Sarcoma of the trachea is slow growing. It may be present for a long time before producing clinical manifestations. The latter are apparently caused primarily by collapse of the wall, due to destruction of the cartilages involved. The exaggeration of symptoms is usually due to increase in the endotracheal growth of the tumor mass itself.

Usually the tumor is situated in the upper third of the trachea and arises from the posterior and lateral walls. It occurs in the earlier decades of life, and its occurrence is equally divided between the sexes. Most of the cases described in the literature have been instances of the spindle cell variety, 2, however, have been cases of myxosarcoma.

The tumor is only locally malignant, metastases being rarely reported and then being present only regionally. Aspiration metastases to the lung and carina were present in the second case reported.

Accordingly, death is not induced by the inherent malignancy of the tumor but rather by a complex mechanism involving the respiratory and cardiovascular systems. The tracheal stenosis produced by these tumors leads to emphysema and subsequently to right ventricular hypertrophy and ultimate cardiac failure.

ALFRED PLAUT, *President**Regular Monthly Meeting, April 27, 1939*ROBERT A. MOORE, *Secretary*

## XERODERMA PIGMENTOSUM D S D JESSUP

A case of xeroderma pigmentosum which had reached the tumor stage is reported. The patient, a boy of 12 years, and his brother two years older, who also has the disease, have been under observation for eight years. They are the only children of their parents, who are not related. The family history is noncontributory. The tumor formations are confined to the face and have been treated by desiccation and trichloroacetic acid, and more recently by excision. Pathologic examinations have shown four squamous cell carcinomas and one basal cell carcinoma. At present the patient's nutrition is good, and there is no anemia. Besides excision of the tumors, treatment has consisted in maintenance on a high caloric diet with cod liver oil and intramuscular injections of liver extract. There has been no evidence of metastasis to the lymph nodes and in considering this disease of infancy and childhood it is interesting to note that the literature does not show any reports of metastasis to lymph nodes from cutaneous carcinoma in cases in which the diagnosis was verified by microscopic examination. Death is usually due to malnutrition and anemia, and, of the 3 patients reported as having come to autopsy, only 1 presented evidence of visceral metastasis, and this was a melanosarcoma of the liver, which was considered secondary to a non-pigmented sarcoma of the face.

The paper will be published in the *American Journal of Cancer*.

## ACQUIRED PARASTERNAL DIAPHRAGMATIC HERNIA ON THE RIGHT RUDOLF A. COLMERS (by invitation)

An obese woman 77 years old died with a clinical diagnosis of cardiovascular disease. There was a history of repeated abdominal pain on the right when the patient was in her forties for which appendectomy and later cholecystectomy were performed without lasting beneficial results. At the age of 57 she underwent an operation for a femoral hernia. A few years later she began to have bronchitis with marked dyspnea and cardiac insufficiency. At this time the first roentgen examination of the chest was made and revealed a peculiar shadow in the right lung field. No definite diagnosis was made, but neoplasm was considered. In the following terminal fifteen years of her life the roentgen findings remained essentially the same. A diagnosis was never established. At no time was there any barium sulfate given to aid roentgen examination. The significant findings at necropsy were as follows:

The diaphragm was at the level of the fifth rib on either side. Behind the xiphoid process and slightly to the right, at the site of the muscle-free space of Larrey, there was a round hole, 5 cm. in diameter, on the under surface of the diaphragm. This hole was the orifice of a hernial sac which extended upward, outward and slightly posteriorly into the right thoracic cavity, reaching the level of the third rib anteriorly. It was entirely covered by pleura. Its anterior wall was formed by rather loose connective tissue, its posterior wall, by the tendinous portion of the diaphragm. Its inner surface was lined by peritoneum. In this hernia, a loop of transverse colon, part of the great omentum and the upper part of the round ligament of the liver were found. The contents of the sac were easily removed and showed no signs of vascular disturbance. The hernia displaced the markedly enlarged heart to the left. There were signs of a healed mild adhesive pericarditis and considerable adipositas. Most remarkable was the middle lobe of the right lung, which was reduced to a thin strip of fleshy tissue. It contained a calcified nodule, apparently a healed primary tuberculous focus. Its bronchus was of normal caliber, but about 1 cm. from its root it showed an

upward kink, and from this point on its lumen was collapsed. Microscopic examination revealed advanced atelectasis and fibrosis, with much coal pigment present. Corpora amylacea were found in some of the alveolar spaces. Elastic fibers were abundant and were present also in the collapsed alveolar walls. The anatomic diagnosis, therefore, is true parasternal hernia on the right, with displacement of the heart to the left. The right middle lobe showed compression atelectasis.

Parasternal hernia on the right is relatively infrequent. According to Hedblom, the instances form about 3 per cent of all cases of diaphragmatic hernia. In the literature there are reports of about 60 cases, many of which include only roentgen findings, recent necropsy reports being relatively rare. The instance reported here is the only recorded case of right-sided parasternal diaphragmatic hernia in which there was shown acquired total atelectasis of the middle lobe of the right lung. This is of importance because of the light that it throws on the causes of the condition.

It is believed that the parasternal diaphragmatic hernia in this case developed during adult life, for the following reasons:

- 1 The patient falls in a group of patients whose age and constitution are generally considered to predispose to parasternal hernia.

- 2 The clinical symptoms referable to the hernia did not appear until the patient was middle aged.

- 3 The many elastic fibers in the alveolar walls of the atelectatic portion of the lung prove that this compression atelectasis developed in extrauterine life.

- 4 The abundant coal pigment in the atelectatic lobe shows that this condition must have been acquired rather late in life, and therefore the time of formation of this hernia narrows down at least to adult life.

As this case in every respect fits into the classic picture of parasternal diaphragmatic hernia and as the time of onset is clear, strong support is lent to the correctness of the theory that such a hernia is acquired in extrauterine life.

#### DISCUSSION

HERBERT J. WIENFR. I should like to compliment Dr. Colmers on his careful preparation and excellent presentation of this case. It is a case I knew very well and followed for a good many years. From the clinical angle it is of considerable interest that when the first roentgenograms of this patient's chest were taken and this peculiar shadow appeared coincident with the clinical symptoms of rather severe bronchitis, it was debated whether there might not be an encapsulated intralobar effusion, and it was suggested and favored by one consultant that a paracentesis be done. Obviously, that would have been an unfortunate procedure, because it would likely have led to an infection of the area punctured. The symptoms really could not be attributed to anything but a pulmonary involvement from the clinical study, which was made by a number of excellent men. No one even considered the possibility of a diaphragmatic hernia.

R. A. COLMERS. I talked over the roentgen findings with Dr. William H. Meyer, our radiologist. He said that it was impossible to make a diagnosis of diaphragmatic hernia from the flat plate but that this condition should be one of the first things to be considered. As diaphragmatic hernia occurs on the right side in only about one sixth or one seventh of all the cases physicians do not generally take it into consideration in making a differential diagnosis. Parasternal hernia, however, is as frequent on the right side as on the left.

#### PULMONARY ATRESIA AND "TETRALOGY OF FALLOT" LOUISE H. MEELER

The case is that of a 54 day old boy whose weight at birth was normal, 92 pounds (4,173 Gm.). There were no obvious abnormalities. The baby was cyanotic from birth and unable to live outside an oxygen tent. There was a history of tremors shortly after birth.



The family history was not important. The patient was the third living child, the 2 others were twins, another child had died on the third day. The patient was admitted to the hospital at the age of 14 days. The color was indigo blue on exertion. There was sucking in of costal interspaces on exertion. No heart murmurs were noted at any time.

The roentgen pictures showed the typical *coccy en sabot* considered by Rossle diagnostic of the tetralogy of Fallot, i. e., apex formed of right ventricle with notch and left ventricle apex above notch, defect in shadow of pulmonary artery and prominence of transposed aorta at right.

The autopsy showed no abnormalities. The lingula of the lung was very large. The heart weighed 58 Gm. The aorta, with three heavy semilunar cusps, was enlarged and in dextroposition, riding over the interventricular defect due to the absence of a membranous septum. There were complete atresia of the pulmonary artery, patent ductus arteriosus and pulmonary arteries dividing above this point. The right ventricle was much enlarged. The left side of the heart was hypoplastic.

Fallot considered that these anomalies constituted a disease entity and were the commonest cause of congenital cyanosis.

PROLONGED HYPERPYREXIA WITH NECROPSY. WARD J. MACNEAL, HENRY H. RITTER (by invitation) and S. MILTON RABSON.

A graduate nurse aged 26 had a surgical revision of the stump of her left index finger on June 21, 1938, for persistent low grade osteomyelitis. This operation was followed by extension of the infection, persistent severe headache, muscular incoordination and gradually rising temperature. Blood cultured on July 8 and 11 yielded a growth of *Staphylococcus aureus*. The temperature reached 106 F at 8 p. m. on July 11. On the next day divided doses of stock staphylococcus bacteriophage were given intravenously to a total amount of 200 cc. At 8 o'clock that evening the rectal temperature reached 113 F, at midnight it had fallen to 99.4. Subsequently the temperature rose again and varied from 104 to 109 for many weeks, with occasional excursions outside these limits up to 110, 111 or even 112. After a period of apparent improvement, the offending finger was disarticulated at its base on September 17. Following this operation the patient failed rapidly, acquired a severe colon bacillus infection of the urinary tract and after several attacks of respiratory failure died, October 9. Necropsy, performed after a delay of twenty-six hours, disclosed marked cerebral edema, pial hemorrhage in the left frontal and parietal areas, marked congestion of the right basal ganglions and of the pons, and in the left side of the cerebellum an irregular cavity occupying the major portion of the site of the dentate nucleus and extending to the right to involve the medial portion of the right dentate nucleus. Microscopically, the lining of the cavity consisted of disintegrating brain substance. Rod-shaped bacteria could be recognized in the brain tissue and were crowded together in its blood vessels. Inflammatory reaction was not recognized, nor could any evidence of a neoplasm be found.

This article will be published in full in the *Archives of Internal Medicine*.

#### DISCUSSION

MILTON HELPERN. I should like to ask Dr. MacNeal how long it was after the autopsy that the brain was sectioned, or whether it was sectioned at the time of the autopsy, because I think the cavities look suspiciously like the postmortem Swiss cheese holes one sees in brains which have remained in solution of formaldehyde for some length of time before sectioning, especially when the post-mortem interval before autopsy is long.

ALFRED PLAUT. Was there any indication of cavities or pseudocavities in other parts of the brain? Many years ago my colleagues and I were confronted with the question of thermometer fraud in a patient. We then simply pushed the thermometer beyond the level of the sphincters, after having attached it to a string, and afterward pulled it out. Even the most skilful faker cannot do anything to a thermometer which is almost in the sigmoid.

WARD J MACNEAL The brain was not sectioned immediately, but at necropsy the lateral ventricles of the cerebrum were opened and the brain was then suspended in solution of formaldehyde. The cavity in the cerebellum was discovered only after fixation for about a week. There was no sign of a similar disintegrating process anywhere else. For that reason we feel that the evidence leaves something to be desired. The importance of this particular observation seems not to have been appreciated at the time. It was only on subsequent study that one realized that here was something of considerable importance. The same criticism holds true in regard to the observations on temperature. In spite of my urging that the temperature should be taken by mouth and rectum simultaneously, this was done for only about four days, and then there was definite rebellion and it was impossible to have it continued. Obviously one's interest in such a case may not be shared by every one concerned.

After a death one is not permitted to do anything to the body without the authorization of the superintendent's office, and although I was regarded as an intimate friend of the patient and her family, and all the members of her family in New York were willing to grant permission for an autopsy, the executive officer was unable to allow anything to be done until the legally responsible member in California had been consulted.

The whole subject is to my mind rather tragic, but if one reads the literature of genuine and fraudulent hyperthermia one finds it has a comical side. There is another paper, dealing with the literature, which will appear along with this one. Temperatures even above 160 F have been reported, as some of the members may know.

#### EXPERIMENTAL TRANSMISSION OF ENDOCARDITIS LENTA WARD J MACNEAL and (by invitation) MARTHA JANE SPENCE and MARIE WASSEEN

Endocarditis lenta, a specific infectious disease of man, has been transmitted to a large proportion of experimental rabbits by repeated intravenous injections of large doses of pure cultures of *Streptococcus viridans* isolated from the blood of human patients. The cardiac vegetations in the rabbits are large and easily recognized by gross inspection, and these lesions exhibit to a large extent the characteristic gross and microscopic features of the human disease. Large colonies of the streptococci are present. Apparently, however, the rabbit possesses a relatively high natural resistance to the infection, the endocardial lesions showing a real tendency to heal. This evident balance between the forces of infection and resistance would seem to make the rabbit a valuable experimental animal in which to study the phases of extension and of healing and also the influence of various therapeutic measures on such processes.

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### BUFFALO PATHOLOGICAL SOCIETY

ERNEST WITEBSKY, *President*

*Joint Meeting with Buffalo Academy of Medicine, Nov 23, 1938*

SAMUEL SANES, *Secretary*

#### SPIROCHETAL JAUNDICE IN BUFFALO NORMAN W ELTON

A fatal case of Weil's disease is presented which was recognized clinically because of the combination of azotemia, jaundice and hemorrhagic manifestations on the sixth day of illness. Death occurred on the tenth day of illness. A white guinea pig inoculated subcutaneously in the groin with urine obtained by catheterizing the patient's bladder on the day of admission to the hospital (sixth day of

illness) died thirteen days later with intense, spectacular jaundice, and multiple foci of interstitial hemorrhage in the lungs. Sections of the liver of this guinea pig prepared with the Levaditi stain exhibited great numbers of typical leptospiras in "c" and "s" forms, having tapered hooked ends. A second white guinea pig was inoculated in the same manner with urine aspirated from the bladder of the first guinea pig at autopsy on the latter animal, and died showing the same gross and microscopic picture on the tenth day.

This case constitutes the eighth reported to date in the United States in which death was proved to be due to leptospirosis icterohaemorrhagica, the third in which the disease was known to have occurred in a fish handler in this country and the first to occur in the Great Lakes port of Buffalo.

This article will appear in full in the *American Journal of Clinical Pathology*

#### PATHOGENESIS OF TUBERCULOUS MENINGITIS K. TERPLAN

For the present discussion 23 cases of diffuse tuberculous meningitis in children, proved to be such by postmortem examination, were selected. In 6 of these tuberculomas were found, either single or in small numbers in different parts of the cerebral cortex, cerebellum and brain stem ganglions, varying in size from that of a lentil to that of a small hazelnut. Only in a single instance did the leptomeninx above the cortical tuberculoma show more pronounced meningeal tuberculosis. In all cases the typical anatomic picture of tuberculous meningitis was present, the exudate being especially dense in the basal cisterns and in the choroid plexus. In the 6 cases in which tuberculomas were found in the brain substance there were also huge hematogenous tubercles in the spleen, liver and lungs. In all cases in which only few miliary tubercles were seen in the liver, spleen or kidneys and almost none in the lungs there were no tuberculomas in the brain substance but rather uniform basal tuberculous meningitis. As in all cases the brain substance was carefully examined for tuberculous lesions, the findings in these cases do not support the view of Rich that in the majority of cases tuberculous meningitis follows direct extension of parenchymatous tubercles into the subarachnoid space. The findings support rather the work of Kment in Ghon's laboratory, who found that tuberculous meningitis with the typical basal localization is always associated with tuberculous lesions in the choroid plexus and with recent tubercles in the leptomeninx. In cases in which tuberculous meningitis had extended from a cortical tuberculoma, the process remained more localized and was most pronounced in the region of the parenchymatous tuberculoma. In those cases the typical picture of diffuse tuberculous meningitis was not present. That the escape of tubercle bacilli into the spinal fluid is most probably secondary to formation of recent tubercles in the leptomeninx had been stressed already by Askanazy and Korteweg, writing almost twenty years ago. The material in the present cases entirely supports the view that diffuse tuberculous meningitis with the classic basal localization of the exudate is either plexo-meningeal or merely meningeal genetically. In addition the material included several instances in which in adults huge tuberculomas were found in the brain substance, infiltrating the leptomeninx, and huge meningeal tuberculomas of plaque-like appearance without evidence of diffuse tuberculous meningitis. In all these instances the plexus was normal and so also was the entire leptomeninx outside the area in which the tuberculomas were found.

#### PURULENT PERITONITIS FOLLOWING APPENDICITIS DUE TO A MEMBER OF THE TYPHOID BACILLUS GROUP, *EBERTHELLA OEDEMATENS*, DE ASSIS ERWIN NETER

Some bacteriologic and immunologic observations are presented from a study of a patient with purulent peritonitis due to *Eberthella oedematis*, de Assis. This seems to be the first report of its kind.

The patient was a girl 18 years old, whose past history was without significance. For two days prior to admission she suffered severe pain in the right lower quadrant of the abdomen, associated with nausea and vomiting. On her admission to the hospital her temperature was 100.2 F and her pulse rate 78. Examination revealed nothing pathologic except rigidity, spasm and rebound tenderness in the right lower quadrant of the abdomen, particularly over McBurney's point. The urine was normal, the blood showed 4,120,000 red cells, with hemoglobin 86 per cent, and 16,000 white cells, with 60 per cent polymorphonuclears, 18 per cent bands and 22 per cent lymphocytes. On operation diffuse purulent peritonitis and a gangrenous appendix without perforation were found. The appendix was removed and the abdomen drained. The patient made an uneventful recovery.

The micro-organism isolated from the peritoneal exudate of the patient on two occasions was a gram-negative motile bacillus, which produced acid within eighteen hours from dextrose, maltose and mannitol. At that time lactose was not fermented. No gas was formed in any of the substances tested. After continued incubation, however, acid (but no gas) was produced also from lactose. Gelatin was liquefied, indol was produced, litmus milk was acidified and clotted. The strain was not agglutinated by either anti-Eberthella typhi or anti-Shigella dysenteriae and paradysenteriae serums. The formation of indol clearly differentiated this micro-organism from Eberthella typhi, and, on the other hand its motility, from members of the Shigella group. The micro-organism isolated from the present patient corresponded to *E. oedematiens* (de Assis, A. Estudos sobre o genero "Eberthella" Buchanan, 1918, Sobre dois novos bacillos pseudotipicos *Eberthella tarda* e *Eberthella oedematiens*, *Bol. Inst. Vital Brasil* 5 3 [Sept] 1928), and was found to be identical in its cultural characters with the two strains of *E. oedematiens* obtained from Dr. de Assis. When rabbits were inoculated intravenously with the strain described (a heat-killed as well as a living suspension), agglutinins readily developed. The antiserum caused large floccules with a formaldehydized suspension and very fine floccules with an alcohol-treated suspension. The serum prepared with the strain described here failed to agglutinate the two strains of de Assis. This finding supplements the observation of de Assis that his two strains were antigenically different.

The patient's serum ten days after the onset of the illness failed to agglutinate the strain of the patient, neither did the serum cause agglutination of the two strains of de Assis. Unfortunately, an examination of the patient's serum at a later time was not possible.

#### FURTHER INVESTIGATIONS ON THE PATHOGENESIS OF HEMORRHAGIC NECROTIC LESIONS IN THE INTRADERMAL PNEUMOCOCCIC INFECTION OF RABBITS E. WITBSKY, E. NETER and H. WARD

There is a close resemblance between the hemorrhagic necrotic lesion occurring in the rabbit infected intradermally with pneumococci virulent for rabbits and the so-called Schwartzman reaction. In the experiments to be described we tried to correlate these two phenomena. The site in a rabbit in which pneumococci had been injected intradermally and which produced swelling and erythema could be transformed within three to four hours into a hemorrhagic necrotic lesion by injecting intravenously an agar-washing filtrate of a culture of *Bacillus typhosus* or *meningococcus* prepared according to Schwartzman's technic.

Pneumococci when examined for their capacity to produce active agar-washing filtrates were found to be negative. Active factors, however, could be obtained, though yet with irregularity, from the pneumococci themselves, especially when autolysates were used. The reason for the irregularity is probably based on the fact that the substance under investigation seems to be very labile as far as temperature and contact with air are concerned.

In our former experiments we were not successful in transforming the site receiving an intradermal injection of Schwartzman toxin or pneumococci, respec-



tively, into a hemorrhagic necrotic lesion by means of an intravenous injection of a suspension of living or heat-killed pneumococci. The difficulties, however, were overcome when pneumococci were grown in 2 per cent dextrose broth. One liter of dextrose broth was centrifuged, and the sediment suspended in about 25 cc of saline solution. If a heavy suspension of pneumococci of that type is used, it is possible to transform a primary lesion of the type described into a hemorrhagic necrotic lesion by means of an intravenous injection of such a suspension. In one series of experiments intracutaneous injections of Shwartzman filtrates (obtained from meningococci) were given, in the second series rabbits were given intradermal injections of pneumococci. Twenty-four hours later a heavy suspension of pneumococci of type I was given intravenously. Within two to four hours the majority of these animals showed a hemorrhagic necrotic lesion at the site of the intradermal injection while the respective controls, not given intravenous injections, revealed no hemorrhagic necrotic lesion whatsoever. The addition of solution of formaldehyde U S P in 0.4 per cent concentration seemed to kill pneumococci rapidly without apparently reducing their capacity to induce the reaction under investigation. While further experiments are necessary in order to obtain more regular results than have been possible so far, the positive observations seem already to add further proof to our original hypothesis, according to which septicemia may be not only a symptom but also an important factor in the pathogenesis of hemorrhagic necrotic lesions of pneumococcic infections.

## PATHOLOGICAL SOCIETY OF PHILADELPHIA

BAXTER L. CRAWFORD, *President*

*Regular Meeting, Dec 8, 1938*

HERBERT L. RATCLIFFE, *Secretary*

### THE ANNUAL GROSS LECTURE FURTHER STUDIES ON THE PATHOGENESIS OF VASCULAR DISEASE M. C. WINTERNITZ

The objectives of the study of vascular disease which my associates and I have carried on for the past three years have included that of a clearer understanding of the structure and function of the blood vessel wall. Especial attention has been directed toward the mural blood supply in arteries and in veins and to the correlation of this with the many manifestations of vascular disease.

The basis for an understanding of the vascular pattern in the blood vessel wall was laid by the embryologic studies of Bremer. The demonstration of this pattern in normal vessels of adults is not possible in all instances, and the pattern varies in different animal species. It is rarely demonstrable in entirely normal arteries of young human beings. However, the vessel wall in its response to injury often demonstrates an abundant vascular network visible because of its contained red blood cells and so arranged as to suggest that it is preformed.

The study of the nutrition of the intimal coat is complicated by the difficulty of establishing a definite norm for the thickness of this layer. However, rapid proliferation of intimal tissue is associated with a rich capillary plexus, the acute processes in the vessel wall which often lead to serious sequelae are accompanied by all the manifestations of exudation that are found in other situations, and the degenerative changes in thickened vessels are found to be associated with obliteration of preexistent vasa vasorum.

The vasa vasorum have three portals of entry into the blood vessel wall, the majority arise in the adventitia from larger branches and penetrate the medial

coat. Another group arise from the intimal surface as direct penetrating branches from the mouths of primary branches of the vessel. These enter the vessel wall and form an anastomotic plexus immediately around the orifice of the branch, from which secondary feeders are given off to the vessel wall in the region. A third group enter the vessel wall from the lumen and anastomose with the branches of the other groups.

With the use of clearing methods, it has been shown that hemorrhage, varying from very fresh and very small extravasations to very old and very extensive accumulations of blood, may be found in any of the coats of the artery and, indeed, of the vein also. Such hemorrhage may be bright red or dark red, orange, yellow and even green. As may be anticipated, there is an associated deposition of iron pigment. The macrophage may be seen in fresh lesions, laden with red blood cells as well as iron. These phagocytes contain much fat, stainable with sudan III. As many of these sudan-stained granules cannot be distinguished from red blood cells, which are also phagocytosed, the question of their relationship arises. A study of this problem has been made by injecting red blood cells as well as other substances into the peritoneal cavities of rabbits under varying circumstances. This work has led to the conclusion that, while macrophages derive their fat content in other ways, the evidence that both iron and fat may be derivatives of red blood cells ingested by them is all but incontrovertible. Confirmation is secured by the study of hemorrhage in other conditions—for example, thyroid adenoma or infarcts. Furthermore, disintegrated blood, in sinusoids within the vessel wall, often seems to provide a nucleus for calcification.

The causes for the variability in the vascularity of the different coats of the artery wall are no more clear than are those for the hemorrhage. The fact that vascularity is present in these coats, however, indicates that the blood vessel wall may react to irritants much as any other vascular tissue does, and that exudation of fluid, cellular elements and fibrin, as well as of red blood cells, may be encountered at different stages of disease of the vessel wall. The conditions which determine hemorrhagic exudate in the wall of the vessel must conform in general to those associated with similar exudate in other tissue.

From time to time one sees examples of disease that suggest strongly that infection plays a role in the production of vascular lesions. Particularly striking have been several cases of endocarditis due to *Streptococcus viridans* which occurred either in children, associated with patent ductus arteriosus, or in adults. In such cases there may occur lesions of the pulmonary artery and aorta, with or without intimal vegetations, which seem to have followed the adventitial vasa vasorum through the vessel wall to the intima. The changes may be both chronic and acute, indeed, it seems that a chronic process may give rise to the vascularity through which the acute process is mediated.

Lesions such as these have great similarity to those produced by *Spirochaeta pallida* and by the virus of rheumatic fever. The question arises whether syphilis and rheumatic disease of the blood vessel wall are as sharply differentiated from lesions due to other agents as has been believed. A boy of 18 years who died after a protracted course of chronic nephritis had focal areas of thickening in the coronary arteries. These were associated with sharp breaks of the elastica and muscularis of the media and vascularization of the vessel wall, extending through the medial coat from the adventitia. Serial examination of coronary vessels quite frequently shows such breaks in the media with more or less cellular infiltration of the adventitia and with thickening of the intima. Anatomic changes of these types, resembling the vascular lesions so characteristic of syphilis and rheumatic fever, make it essential to seek in infection, or perhaps, as a corollary, in allergy.

To illustrate the possible role of infection in lesions of the vessel wall, a series of experiments was devised. In the first place, it was found that the normal

femoral artery of man could be injected via the lumen of the neighboring vein. With this established, into the walls of veins of goats was injected a minute quantity of one of several organisms: *Staphylococcus aureus*, *Streptococcus viridans*, or a strain of *Bacillus pyocyaneus* isolated from a necrotizing intestinal arteritis encountered at the postmortem table. The extent of the lesion was determined after varying intervals.

The lesions in the wall of the vein varied from acute phlebitis, with or without thrombosis, to fibrous intimal plaques, depending on the organism used as well as on the duration of the experiment. Changes in the neighboring artery consisted of intimal proliferation, which was often quite rapid, exudation, with precipitation of fibrin in the vicinity of the internal elastic lamella, and, later, dense fibrous intimal thickening. Although in most cases the injection was made into a loose vascular tissue surrounding both artery and vein, it was sometimes possible to demonstrate what was apparently a vascular passageway for the infection from vein to artery. A lesion of the femoral vein and artery closely resembling these experimental lesions was encountered recently in a man at autopsy.

## Book Reviews

**Etude morphologique et biologique sur les flagellés intestinaux parasites des Muridés Etude comparative des flagellés du Cobaye** By Leon Morenas Pp 234 Price, 60 francs Paris Masson & Cie, 1938

In this monograph on the parasitism of the Muridae by intestinal flagellates the emphasis is on the biologic rather than on the morphologic studies. The hosts studied were *Epimys norvegicus*, *Epimys rattus* (*rattus* and *Alexandrinus*), *Mus musculus*, *Apodemus sylvaticus*, *Pitymys subterraneus*, *Pitymys duodecimcostatus*, *Arvicola amphibius* and *Cavia porcellus*. The genera *Oikomonas*, *Sphoeromonas* and *Selenomonas* (of the *Monadidae*) are considered to be coprozoic, whereas the genera *Monocercomonas*, *Trimitus* and *Eutrichomonas* are thought to be young or transitional forms of *Trichomonas*. *Enteromonas* is considered to represent true parasites. Two new species of trichomonads are described: *Trichomonas guartii* of the rat (which has been previously considered a *Retortamonas*) and *Ditrichomonas lavieri* from *Pitymys subterraneus*. A *Trichomonas* identical with *Trichomonas parva* of the rat was found in the field mouse, *Arvicola amphibius*, a rodent widely different from the rat but of similar habits. *Chilomonas intestinalis* was observed in the guinea pig and *Chilomonas bettencourtii* in rats and mice, and *Chilomonas caviae* was found again in the guinea pig and restudied. Among the *Diplomonadidae*, *Hexamita muris* and *Syndyomita intestinalis* were observed. The only new finding in connection with the *Giardia* is that the dwarf form of *Giardia microti* (from the field mouse) constitutes a new species.

Although the comparative frequency of infections in laboratory rats was higher than in sewer rats, the parasitic index in the latter was higher than has been generally admitted. Wild mice are parasitized only exceptionally, whereas Muridae of the fields (black rat, field mice) are parasitized nearly uniformly. In the latter case, however, the parasitism is usually single in an individual host. The appearance of parasitism in the young corresponds with the beginning of the weaning period and is correlated with the modifications of the intestinal flora. *Hexamita* and *Giardia* appear more precociously than *Trichomonas* and *Chilomastix* because of being less influenced by dietary modifications.

The conditions which determine the parasitism are the  $pH$ , the diet and the flora. A progressive alkalinity following the small intestine was found to drop abruptly to acidity in the cecum. With the exception of *Chilomonas* (which requires an alkaline cecal content), the parasitism adapted itself so well to different values of  $pH$  as to indicate that the latter plays only a minor determining role. Casein, the only protein in the diet, had a very unfavorable action on the development of *Trichomonas*. Whereas avitaminosis C in the guinea pig was without effect on the flagellate fauna, avitaminosis A in the rat brought about diminution and sometimes disappearance of the flagellates. This modification was thought to act through changes in the metabolism of mucus as well as in the  $pH$  and the intestinal flora. Ratchiffe's observation that the existence of cecal flagellates seems to be incompatible with a flora rich in gram-positive bacteria was confirmed. This was supported also by cultural findings. The author believes that most of the flagellates in the Muridae and especially those in the *Caviidae* should be thought of as inquilines rather than as true parasites.

**Les groupes sanguins Leur application à la biologie à la médecine et au droit** Professeur Ludwik Hirszfild, directeur du département de bactériologie et médecine expérimentale de l'Institut d'Hygiène de l'État, à Varsovie. Translated from Polish by Mme Hanna Hirszfild. Paper. Pp 169, with 16 illustrations. Price, 30 francs. Paris Masson & Cie, 1938.

The author of this book has played a significant part in the development of the knowledge of blood groups. Together with von Dungern, he worked out a hypothesis of inheritance of blood groups, a part of which is still valid, though another part was supplanted by the hypothesis of Bernstein. Von Dungern and



Hirszfeld discovered the subgroups  $A_1$  and  $A_2$ . Later, during the war, Hirszfeld and his wife, the translator of the book, discovered the differences in the ethnic distribution of blood groups and thus initiated a new advance of research in anthropology.

The author states in the introduction that he wishes to be like the experimenter in attention to details and like the architect in seeing with the eyes of the mind the edifice of the future. He is singularly qualified to do both.

The book is intended for physicians, biologists and lawyers. It avoids technical details and can be easily understood by those who have a basic knowledge of the biologic sciences.

The first chapter, entitled "The Individuality of Blood," is a simple and clear introduction to the subject matter. It includes a historical review.

The following five chapters take up the inheritance of the group properties, the subgroups  $A_1$  and  $A_2$ , the newer concepts of group O and the M, N, P, X and Q factors. Then follow a chapter on the exclusion of paternity and another particularly interesting one on the exclusion of maternity. The distribution of group factors in organs, secretions and tumors is treated in a separate chapter. In the discussion of the application of blood groups in criminologic investigation Hirszfeld stresses the sources of error in the interpretation of blood stains. In the succeeding brief but meaty chapters the following topics are dealt with: the blood groups in animals, the transmission of agglutinability (the so-called Thomsen phenomenon), destruction by enzymes, the role of the thermal amplitude, the concept of serologic constitution, the ethnic distribution and hypotheses concerning the appearance of blood groups. A touch of humor is introduced in the brief fourteenth chapter, on erroneous applications of the knowledge of blood groups to racial theorems which serve political purposes by appealing to national vanity. In the final chapter the usual view of the blood groups as static is replaced by a concept of dynamic gradations in the quantities of the blood group factors, the decrease of the A factor, for example, being accompanied by a parallel growth of property O. The role of mutations is presented convincingly.

The clarity of the presentation of complex problems, the synthetic and philosophic approach, the mastery of the handling, the emphasis on the essentials and the brevity make the reading of this book an exhilarating experience. It is heartily recommended to all who are interested in the subject.

**Cancer with Special Reference to Cancer of the Breast** R. J. Behan, M.D., Dr. Med. (Berlin), F.A.C.S., Cofounder and Formerly Director of the Cancer Department of the Pittsburgh Skin and Cancer Foundation, Pittsburgh. Cloth, Pp. 844, with 168 illustrations. Price, \$10. St. Louis: C. V. Mosby Company, 1938.

According to the preface, "this book is written primarily for the clinician who is seeking to enlarge his knowledge of the cancer problem." It is intended especially for the practitioner of medicine "whose practice is limited and whose collateral reading is not sufficiently extensive to familiarize him with the more important advances of cancer research and cancer treatment." It was originally written as a treatise on cancer of the breast, but since a comprehensive knowledge of cancer in general is required for the understanding of cancer of any single organ, it was extended to include consideration of many phases of cancer. The first 14 chapters (452 pages) are devoted to cancer in general, with special reference to breast cancer, and the remaining 15 chapters (361 pages), to treatment in various forms. The book is a compilation of the literature on cancer and not an authoritative presentation of well digested knowledge of the nature and of the diagnosis and treatment of cancer. References to the sources abstracted are given at the bottom of the page. The two chapters on the etiology of cancer, especially cancer of the breast, carry no less than 454 references, the chapter on pathologic physiology 379 and the chapter on irradiation 320. These figures indicate the scope of the compilation. The book contains a vast amount of facts and opinions about various aspects of cancer and no doubt will be of service, but mainly as a guide or index to original sources of information.

## ACUTE POSTOPERATIVE ESOPHAGEAL, GASTRIC AND DUODENAL ULCERATIONS

A FURTHER STUDY OF THE PATHOLOGIC CHANGES IN SHOCK

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AND

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In a previous study in which we investigated the pathogenesis of acute postoperative enterocolitis we arrived at the conclusion that the mechanisms involved in the production of that condition were those which formed the bodily responses to shock<sup>1</sup>. In several instances we were impressed with the occurrence of acute duodenal, gastric and esophageal erosions or ulcers simultaneously with typical enterocolitis. We therefore decided to study a group of cases in which these esophageal, gastric and duodenal lesions were present, in order to determine whether their occurrence in association with enterocolitis was merely a coincidence or dependent on the same vasomotor mechanisms for their causation.

### HISTORICAL REVIEW

The earliest report of a case of postoperative duodenal ulceration was communicated by Billroth<sup>2</sup> in 1867. He presented the case of a 42 year old man with an enormous substernal goiter which almost caused suffocation. The man's general condition was so poor that Billroth hesitated to operate and hence attempted to evacuate the fluid contents of the cystic gland. Since this was not successful, he operated and exposed the gland by incising the fascia over it so that the gland protruded. Owing to the condition of the patient and the fact that the gland extended substernally, nothing further was done. Twenty-four hours later there was abdominal pain, diarrhea, profuse perspiration and restlessness. Two days later the patient was in collapse, and on the fourth day it was noted that his bowel movements were tarry. He died.

From the Laboratories of the Mount Sinai Hospital

1 Penner, A., and Bernheim, A. Arch Path **27** 966, 1939

2 Billroth, C. A. T. Wien med Wchnschr **17** 705, 1867

two days after this, with a terminal rise in temperature. At autopsy there were observed four acute ulcers in the duodenum. In his report Billroth mentioned that he had been told of 2 other cases, one of these was identical with his own, the other was a case of inguinal hernia which had become strangulated and was operated on after fourteen hours of vain attempts at reduction. At autopsy in the latter case several hemorrhagic infarcts were encountered in the stomach and two gastric ulcers symmetrically arranged with relation to the lesser curvature. Despite the fact that there were no clinical signs of sepsis and that in his own case he specifically noted that the wound was clean, Billroth considered that the lesions were embolic in origin and probably septic.

Twenty-two years later von Eiselsberg<sup>3</sup> reported a series of 8 cases of gastroduodenal bleeding occurring after operation. Of the patients, 3 survived. These patients had enormous hemias, which were finally reduced operatively. The fourth case was one of carcinoma of the rectum, which was resected. The patient went into collapse forty-eight hours after operation and died three days later. Autopsy showed diffuse peritonitis, there were also about 30 fresh hemorrhagic erosions in the stomach, of which 2 were about the size of a dime. The ulcers showed "nothing specific." His other cases were similar in nature and concerned incarcerated hemias. He considered the lesions to be the result of retrograde emboli from thromboses in the omentum. His interest in this subject persisted and was evidenced by the papers of Busse<sup>4</sup> and von Winwarther<sup>5</sup> from his clinic. These papers were concerned mainly with the clinical features of postoperative intestinal hemorrhage. Their main importance lies in the fact that they record observations on cases in which the intestinal tract was not operated on. Thus von Winwarther noted these lesions occurring after splenectomy and after operations for ectopic pregnancy, hypernephroma and retroperitoneal sarcoma. Despite this, they affirmed the etiologic significance of retrograde embolism.

Dieulafoy, who is frequently credited with the first description of postappendectomy gastroduodenal ulceration<sup>6a</sup> noted the clinical occurrence of hematemesis after appendectomy. Although he made no post-mortem observations in these cases, he considered the process identical with that observed in a case of gastric ulceration after strangulated hernia, in which he studied the gross and microscopic lesions. He grouped these with instances of "toxic-infectious" gastric ulcerations in which he had found pneumococci<sup>6b</sup> present in the lesions and with

3 von Eiselsberg, F. Arch f klin Chir **59** 837, 1899

4 Busse, W. Arch f klin Chir **76** 122, 1905

5 von Winwarther, J. R. Arch f klin Chir **95** 161, 1911

6 Dieulafoy, G. (a) Presse med **9** 73, 1901, (b) Gastrite ulcereuse pneumococcique, in Clinique medicale de l'Hotel-Dieu de Paris, Paris, Masson & Cie, 1899, vol 3, (c) Exulceratio simplex, *ibid*, vol 2, p 1

his "exulceratio simplex" <sup>6c</sup> His clinical descriptions leave little doubt as to the presence of shock in his cases

In 1902 Nietzsche <sup>7</sup> reported an interesting case of gastric hemorrhage occurring after appendectomy The patient had been ill with peritoneal symptoms for three days before coming under observation He was delirious, presented marked distention and regurgitation of coffee ground material, and had a weak pulse He was not operated on At autopsy generalized peritonitis was found No mesenteric thromboses were observed The stomach showed countless flat ulcers from pinhead to pea size distributed in the fundus and along the greater curvature and covered with clotted blood Microscopically the mucosa appeared well preserved in the areas which were not grossly ulcerated Throughout the stomach the vessels and capillaries of the submucosa were focally distended with blood Occasionally, normal areas showed thrombosed venules in the submucosa In addition, he observed focal accumulations of leukocytes—usually noted in otherwise normal areas of the mucosa, less often in the submucosa Furthermore, he noted focal areas in which the free ends of the tubules showed necrosis These focal necroses were interspersed between well preserved ones He considered the lesions to be due to sepsis

Similar lesions have been observed in the esophagus (Kaufmann,<sup>8</sup> 1909, Pringle, Stewart and Teacher,<sup>9</sup> 1921) In the series reported by Pringle, Stewart and Teacher the esophageal lesions occurred following postappendical peritonitis six times, three times subsequent to cholecystectomy for cholecystitis and pancreatitis and once as a result of a traumatic rupture of the liver with intra-abdominal hemorrhage One instance was noted in which the lesion was found at autopsy following death from peritonitis due to perforation of a duodenal ulcer In the opinion of these authors the lesions were the result of antemortem digestion of the esophagus following regurgitation of gastric contents They expressed great surprise at the observation in their case of traumatic rupture of the liver with marked engorgement of the mucosal vessels and blood pigmentation of the tissues but no cellular reaction, findings which they attributed to the rapid collapse and death of the patient Their protocols leave no doubt of the presence of shock in their cases

More recently Bartels <sup>10</sup> was able to find 82 such cases in his study of a series of 6,000 esophageal specimens Of these, 55 were post-operative He noted that in these cases "it might be said that all the

7 Nietzsche, E Deutsche Ztschr f Chir **64** 180, 1902

8 Kaufmann, E Lehrbuch der speciellen pathologischen Anatomie, ed 5, Berlin, G Reimer, 1909, p 388

9 Pringle, J H, Stewart, L T, and Teacher, J H J Path & Bact **24** 396, 1921

10 Bartels, E C Arch Path **20** 369, 1935

operations were rather difficult and extensive, and occasionally, the patients constituted poor surgical risks" Of the remaining 27 cases there were 13 of sepsis in which embolic phenomena could not be ruled out In addition, there were 6 instances of chronic ulcerative colitis with severe debilitation, and, most interestingly, 1 instance of acute coronary thrombosis in which death occurred in twenty-four hours He notes that "it seems necessary that the patient should be debilitated, but not necessarily dying before changes can occur in the esophagus" He attributes the lesions to the action of regurgitated gastric juice

It is well known that about 45 per cent of instances of *melena neonatorum vera* are due to ulceration in the esophagus, stomach or duodenum (Shukowsky<sup>11</sup>) The pathogenesis of such ulcerations is obscure, but their presence in infants on the first day of life (Zadek<sup>12</sup>) makes a peptic factor unlikely Individual case reports with complete autopsy protocols are not numerous However, Pomorski<sup>13</sup> in 1892 reported the case of a premature child, born after a normal labor and apparently normal, who began to vomit blood two days after birth, went into collapse and died Necropsy showed a large hemorrhage in the fourth ventricle with compression of the cerebellum He also noted the remarkable hyperemia of the gastric and duodenal mucosa and the associated marked edema and focal hemorrhages Microscopic study showed the marked edema of the mucosa and submucosa with hyperemia and punctiform mucosal hemorrhages In addition, there was an ulcer in the cardiac end of the stomach which extended into the submucosa, as well as several punctiform hemorrhages in the small intestine Similar conditions have been described by Vassmer,<sup>14</sup> Zadek,<sup>12</sup> Spiegelberg<sup>15</sup> and von Preuschen<sup>16</sup> as resulting from intracranial birth trauma

The occurrence of such acute erosions and ulcerations in the stomach and duodenum has been repeatedly noted in consequence of severe cutaneous burns (Curling<sup>17</sup>) Harkins<sup>18</sup> presented a historical review of this subject and referred to a report by Swan in 1823 as the earliest record of such a case Furthermore, similar lesions have been noted as occurring in instances of prolonged exposure to cold and resultant freezing (Adams<sup>19</sup>, Forster<sup>20</sup>) Despite the common opinion that these

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11 Shukowsky, W P Arch f Kinderh **45** 321, 1907

12 Zadek, I Arch f Verdauungskr **18** 785, 1912

13 Pomorski, J Arch f Kinderh **14** 165, 1892

14 Vassmer Arch f Gynak **89** 275, 1909

15 Spiegelberg, cited by Landau, L Ueber Melaena der Neugeborenen, nebst Bemerkungen über die Obliteration der foetalen Wege, Breslau, Maruschke und Berendt, 1874

16 von Preuschen, F Centralbl f Gynak **18** 201, 1894

17 Curling, T B Med-Chir Tr London **25** 260, 1842

18 Harkins, H N Surgery **3** 608, 1938

19 Adams, S Am M Times **6** 101, 1863

20 Forster, A Mitt a d path anat Anstalt Wurzburg **2** 155, 1861

lesions are the result of post-traumatic sepsis it should be pointed out that the work of Underhill and his co-workers<sup>21</sup> as well as that of Davidson and Matthew<sup>21a</sup> indicates that the vascular changes as well as the alterations in the concentration of the various components of the blood are identical with those which occur in shock produced by other means. Similarly Haikins<sup>18</sup> has shown that blood concentration begins almost immediately after freezing has occurred, with a rise in hemoglobin values to 168 per cent, and only later leads to the fatal drop in blood pressure. Furthermore, in the cases reported by Adams<sup>19</sup> and Forster<sup>20</sup> there were no evidences of sepsis either clinically or at autopsy.

The acute ulcers and erosions of the esophagus, stomach and duodenum described by Cushing<sup>22</sup> and related by him to lesions in the interbrain or its various pathways appear to us to belong in this category. In analyzing his 11 case reports we excluded from our present consideration 2 cases (6 and 7) in which diffuse vascular disease was present (malignant hypertension) and 2 cases (10 and 11) in which chronic ulcers were observed. Analysis of the remaining 7 cases shows that the lesions followed intracranial operations. Most of these operations were for tumors located in the cerebellum, but one was for a tumor in the olfactory groove and one for an aneurysm of the basilar artery. Similarly the tumors varied in type, among them were included astrocytoma, medulloblastoma, angioblastoma and meningioma. In contrast to these variable factors we note the almost invariable large loss of blood in intracranial operations, which may amount to 500 to 900 cc in a simple craniotomy and exploration and to over 2,000 cc in removal of a large meningioma (White and co-workers<sup>23</sup>). Another accompaniment of craniotomy is the drop of intracranial pressure which occurs when the dura is incised. This leads to overactivity of the carotid sinus, resulting in vasoconstriction in the viscera and increase in the secretion of epinephrine (Ask-Upmark<sup>24</sup>). These are all components of the picture of shock.

#### REPORT OF CASES

In a survey of the postmortem records of the Mount Sinai Hospital for a period of approximately ten years we found 47 cases in which

21 Underhill, F. P., Carington, G. L., Kapsinow, R., and Park, G. T. *Arch Int Med* **32** 31, 1923.

21a Davidson, E. C., and Matthew, C. W. *Arch Surg* **15** 265, 1927.

22 Cushing, H. *Peptic Ulcer and the Interbrain*, in *Papers Relating to the Pituitary Body, Hypothalamus and Parasympathetic Nervous System*, Springfield, Ill., Charles C. Thomas, Publisher, 1932.

23 White, J. C., Whitelaw, G. P., Sweet, W. H., and Hurwitt, E. S. *Ann Surg* **107** 287, 1938.

24 Ask-Upmark, E. *Acta psychiat et neurol*, 1935, supp 6, p. 1.

necropsy revealed the presence of acute gastric or duodenal ulcerations or erosions either alone, together or in combination with acute ulcerative or diphtheritic esophagitis, enteritis or colitis. In selecting these cases we did not include any in which there was present a diffuse vascular disease, such as periaenteritis nodosa, malignant nephrosclerosis or chronic glomerulonephritis with uremia. Further, we did not include any case in which an operation had been performed on the stomach or duodenum. We also excluded from our consideration cases of definite sepsis and cases in which chronic gastric or duodenal lesions were present. There were clinical and pathologic data for study in 37 cases, but the histologic material was necessarily scant and of value in only 18 cases. Four cases are reported in detail to illustrate our findings.

CASE 1—H. E., a 49 year old man, was admitted to the medical service of Dr. George Baehr with a four months' history of a gnawing pain in the left upper quadrant of the abdomen, postprandial diffuse abdominal cramps, and anorexia, accompanied by infrequent nausea but no vomiting. During this period there had been noted also progressive weakness, increasing dyspnea and a loss of 14 pounds (6.4 Kg.) in weight. A guaiac test of the stool was 3 plus, the hemoglobin content was 80 per cent, the blood pressure was 130 systolic and 90 diastolic. An electrocardiogram showed changes indicative of left ventricular hypertrophy with myocardial damage and intraventricular block. A barium sulfate enema revealed an irregular stenosing neoplasm in the transverse colon. The patient was transferred to the surgical service of Dr. Ralph Colp, where an exploratory laparotomy revealed inoperable carcinoma of the hepatic flexure, with metastases to the liver and the tail of the pancreas. Palliative ileosigmoidostomy was performed, and the ileum was divided and sutured about 6 inches (15 cm.) proximal to the ileocecal valve. The first day after operation the patient's temperature rose as high as 104 F., and generalized abdominal tenderness developed. His blood pressure was 168 systolic and 70 diastolic. Coarse rales appeared in the lower lobes of both lungs. On the fourth postoperative day the abdomen became markedly distended, and drainage from the Levin tube was noted to have a fecal odor. The patient became cyanotic. An electrocardiogram taken at this time showed only slight changes. He rallied slightly for a short time under oxygen therapy, then he gradually became more cyanotic and died on the sixth postoperative day.

*Postmortem Observations*—The peritoneal cavity contained about 450 cc. of dirty brown, turbid fluid. The small intestinal loops were irregularly agglutinated and covered by greenish friable material. All the operative suture lines were intact. The small intestine proximal to the anastomosis was enormously distended. The serosa of the jejunum showed occasional areas of reddening with pinhead-sized hemorrhagic dots. The stomach was distended. The mucosa of the fundus and along the magenstrasse was reddened, and there were a number of hemorrhagic dots and pinhead-sized erosions over the posterior wall, a few also were present along the upper part of the magenstrasse. On the anterior wall of the fundus there were innumerable superficial erosions from 2 to 4 mm. in diameter, with a moderate number ranging up to 6 to 7 mm., the latter being quite red. The duodenum was distended. About a foot from the duodenojejunal flexure the mucosa became slightly reddened and covered, especially at the base of the rugae,



*A*, focal necrosis in the mucosa of the fundus of the stomach extending down to the muscularis mucosae (case 1) In addition, interglandular edema is shown, most prominent near the free border of the mucosa *B*, section through the edge of an esophageal erosion, showing the sharp line of demarcation as well as the pronounced vascular engorgement and submucosal edema (case 4)



by a pseudomembrane of yellowish granular friable material. This change was at first somewhat scattered but soon became extensive, with the necrotic material reaching to the apex of the folds. In places there were necroses of the apices of the folds. There were two such areas about 6 inches (15 cm) long with an intervening area of mucosa of about equal length where the jejunum showed nothing remarkable. About 6 inches beyond the end of the second necrotic stretch there was a small area of reddening of the mucosa with erosion of the apices of the folds. The rest of the gastrointestinal tract was without other significant changes except for the carcinoma located at the hepatic flexure of the colon. Additional findings included fibrosarcoma of the tail of the pancreas and hilus of the spleen, with metastases to the liver, and bronchopneumonia.

Microscopic examination of the stomach through an erosion showed primarily mucosal alterations. There was a focal area of necrosis extending down to the muscularis mucosae, in which the glands were destroyed and the tissue consisted of an eosinophilic mass with nuclear debris and red blood cells. Close to this, in the mucosa, was a focal area of hemorrhage. In addition, the interglandular stroma appeared widened in places, with some edema fluid present. This was especially noticeable near the free borders of the glands. The underlying submucosa showed no significant changes. The involved portion of the jejunum showed striking dilatation of the vessels of the submucosa, where venous and capillary congestion was also striking. Some edema was present. The vessels between the muscle bundles in the muscle coats were blood filled. There were, in addition, scattered areas of necrosis, confined mainly to superficial portions of the mucosa. In focal areas, however, this necrosis extended through the mucosa, and in one area there was ulceration extending to the depths of the muscle layer. In the areas where the ulceration was extensive, there was a considerable cellular reaction, primarily of lymphocytic cells.

CASE 2—S K, a 70 year old woman, was admitted to the medical service of Dr George Baehr. She was known to have had hypertension for two years, with progressive dyspnea, precordial pain and weakness. For three days before admission she had experienced anorexia, headache, vertigo, insomnia and rapid respirations. On admission she was dehydrated, with poor turgor of the skin and softness of the eyeballs, the blood pressure was 150 systolic and 70 diastolic, and the hemoglobin content 107 per cent. She showed moderate cyanosis and marked peripheral sclerosis. Her diabetes was controlled by a diet of 80 Gm of carbohydrate, 60 Gm of fat and 40 Gm of protein with the daily doses of insulin of 10-0-10 units and the acetoneuria present on admission disappeared. Despite this, her temperature rose to 105.2 F, intravenous administration of fluids was of no avail, she became progressively more dehydrated and died three days after admission. A 25 per cent solution of pyridine betacarboxylic acid diethylamide (coramine), 3 cc, and epinephrine hydrochloride 7 minims (0.061 cc) were given as a last resort.

*Postmortem Observations*—The peritoneal surfaces were everywhere gray, smooth and glistening. There was no free fluid. The esophagus showed some mucosal congestion, and for about 5 cm above the cardia it was greenish black along the longitudinal folds. The stomach was markedly dilated. It was studded with numerous slightly depressed round black areas, varying from 1 to 2 mm in diameter. About 3 cm below the cardia there were two slightly depressed mucosal defects, round in outline, each 4 mm in diameter, with a smooth gray base and surrounded by slightly hemorrhagic mucosa. In the antrum, immediately above the pylorus, there were several mucosal defects as described for the cardia, but

slightly larger, some of which had a hemorrhagic base. The first portion of the duodenum was intensely congested. There were several black areas, serpiginous in outline, varying from 0.5 to 2.5 cm in diameter. In addition, there were some mucosal defects as described for the stomach. The rest of the gastrointestinal tract was without significant change.

Microscopic examination of the duodenum showed widespread necrosis of the mucosa with destruction of glands and diffuse leukocytic infiltration. It showed a submucosa markedly widened by deposition of fibrin and edema fluid, hemorrhage and leukocytic infiltration. The muscular layers were substantially without change except for congestion of the smaller vessels, more intense toward the submucosa. One larger vein in the muscularis showed an organized mural thrombus. In the area where the mucosa was completely necrotic and the submucosa showed the aforementioned changes, Brunner's glands were uninvolved. Sections taken from the stomach showed severe postmortem changes. In addition, there were focal areas of hemorrhage in the mucosa with interglandular edema fluid.

CASE 3—S. G., a 36 year old man, had been in the hospital before, having been discharged about one month prior to this admission, at which time a diagnosis of intramedullary cord disease was made. He reentered the neurologic service of Dr. Israel Straus because of progression of his symptoms, particularly weakness of the left arm. On his previous admission it was determined that he had no block, and the spinal fluid showed no increase in protein. No gross tumor or disease of the cord was found on exploratory laminectomy of the cervical region. An infection of the wound developed. About four weeks after operation his temperature rose steadily, and meningeal signs were manifest. He died with a picture of meningitis and hyperpyrexia, having been febrile from the day of operation to death.

*Postmortem Observations*—The peritoneal surfaces were smooth and glistening, and the cavity was free from fluid. The intestines were moderately distended. The stomach was slightly congested. On the posterior surface of the duodenum, about 3 cm from the pyloric ring there was a circular fiery red area, about 1 cm in diameter, slightly depressed below the surface. The margins of the ulcer were firmer than the surrounding tissue. About 2 cm below this, on the posterior surface of the duodenum, there was another fiery red depressed area, about 3 cm in diameter. In this case, however, there was no evident marginal induration. A few centimeters below this, at the level of the papilla of Vater, there was a small fiery red area running transversely to the duodenum along the summit of one of the mucosal folds. There was no area of induration. The remainder of the gastrointestinal tract showed no significant changes. The primary neurologic lesion was found to be a parietal dural endothelioma on the right.

Microscopic examination of the duodenal lesion showed a focal area of mucosal necrosis with underlying hemorrhage and focal polymorphonuclear reaction. Brunner's glands in this region were partially maintained and only focally destroyed. The submucosa in this area was markedly edematous, without congestion. Occasional small vessels contained eosinophilic granular masses. The external layers were unchanged. There was no evidence of chronic inflammatory reaction.

CASE 4—D. de C., a 40 year old Italian laborer, was admitted to the surgical service of Dr. Richard Lewisohn with a history of onset of generalized abdominal cramps two weeks previously with intermittent vomiting. The day after onset the pain localized in the right lower quadrant of the abdomen. There was low

grade fever for a week before admission. The patient did not appear acutely ill. The abdomen was soft. There was a mass the size of a sausage in the right lower quadrant of the abdomen, slightly tender, and there was slight resistance in the right subcostal region anteriorly. The blood pressure was 110 systolic and 70 diastolic. The white blood cell count was 9,300, with 70 per cent polymorphonuclears. The hemoglobin content was 92 per cent. Two days after admission laparotomy was performed with the patient under spinal anesthesia, and a retrocecal and retrocolic abscess was opened and drained. It contained two fecaliths. Postoperatively the patient was persistently distended in spite of a Levin and a rectal tube. On the fourth day after operation he eviscerated subcutaneously, and the bowel had to be reduced. Thereafter he did poorly. The pulse rate remained almost constantly at 120 or over, the temperature gradually mounted to 105.4 F, and death occurred three days later, with signs of spreading bronchopneumonia and diffuse peritonitis.

*Postmortem Observations*—The abdomen was distended. No jaundice, petechiae or edema was noted. The appendical drains entered a walled-off abscess cavity, about 7 cm in diameter, containing about 20 cc of fairly thin slightly sanguineous yellow fluid. The small intestine was markedly distended, its surface was slightly dulled and covered with occasional deposits of fibrin. There was a small amount of slightly turbid yellow fluid in the abdominal cavity. The esophagus showed in its lower half large areas of mucosal denudation and areas where the mucosa was replaced by longitudinal layers of dull yellow membranous material. The cardiac portion of the stomach showed, especially along the lesser curvature, irregular areas where the mucosal surface was dulled and granular. Near the cardioesophageal junction were small zones of mucosal roughening with small mottled purple-brown polypoid excrescences, measuring 0.2 to 0.5 cm. The remainder of the stomach was unchanged. The small intestines were markedly distended and contained a large quantity of thin brownish fluid. The mucosa was smooth, pale and glistening. The cecum was distended. The appendix was inflamed and at the tip there was a perforation leading into the abscess cavity described. The large intestines and the rectum were unchanged.

Microscopic examination of the esophageal lesion showed edema and enormous congestion extending through the entire submucosa into the mucosa, with focal hemorrhages in the mucosa. The muscularis also showed marked vascular congestion. There were focal areas in which the squamous epithelium was replaced by a necrotic fibrinopurulent pseudomembrane, beneath which the capillaries contained hyaline thrombi. The cardiac glands were uninvolved.

The histologic observations described in the esophageal and duodenal lesions are strikingly similar to those previously described in our study of acute postoperative enterocolitis. The earliest lesion appears to consist in marked distention of the capillaries and venules, first in the submucosa and subsequently in the mucosa. Next one finds focal hemorrhages in relation to distended vessels, then focal mucosal necroses. These spread and fuse, and where they are extensive they may penetrate the deeper layers of the wall and be accompanied by inflammatory cellular reactions. Hyaline thrombi are irregularly observed in the smaller vessels. Focal sparing of the gastric glands in involved areas of the lower portion of the esophagus and of Brunner's glands in the duodenum was noted.

The histologic pictures of the gastric erosions and ulcerations appear to be different. The earliest changes are observed in the mucosa, where vascular congestion and subsequent focal hemorrhage, initially more prominent toward the base of the glands, near the muscularis mucosae, are seen. Widening of the interglandular stroma is observed, and the presence of mucosal edema. Focal mucosal necroses appear, unaccompanied by any significant cellular reaction. More extensive necroses may penetrate the deeper layers of the wall and be accompanied by an inflammatory cellular reaction. With the early or superficial lesions, the submucosa, muscularis and serosal layer may be without recognizable changes. The observation that the gastric changes begin in the mucosa itself may be attributed to the fact that the most peripherally located arterial plexus in the stomach lies in the mucosa and just within the muscularis mucosa (Djörup<sup>25</sup>), while in the esophagus and in the small and large intestines this plexus is located in the submucosa (Patzelt<sup>26</sup>).

#### COMMENT

The focal destruction of the mucosa which we previously noted in the ulcerative enterocolitides is observed to involve the esophagus, stomach and duodenum in the cases presented here. As in the ulcerative enterocolitides, these lesions are here found in association with a wide variety of "primary" conditions. Among the latter are acute appendicitis with peritonitis, thoracotomy for pulmonary gangrene and for pulmonary abscess, cholecystectomy with pericholecystic abscess, suprapubic prostatectomy, diabetic acidosis, laminectomy with meningitis, and other conditions. In addition to the "primary" conditions which occurred in our own series we should note that identical lesions have been observed in the newborn in association with birth trauma, as well as in persons suffering from burns or freezing or from intracranial operations (see earlier comment on this).

As indicated, this wide variety of primary conditions have in common the factor of shock, whether this is due to peritonitis, diabetic acidosis, hemorrhage, trauma or operation. The physiologic responses to the process of shock regardless of its cause have been discussed in our previous paper. We may briefly summarize them as follows. The vascular response to the diminution in blood volume which characterizes shock consists in arteriolar contraction. The duration of this reaction depends on the duration of the application of the shocking process and on the accumulation of local metabolites, which have the property of causing vasodilatation. The anoxemia resulting from the vasoconstrict-

25 Djörup, F. *Ztschr. f. Anat.* **64** 279, 1922.

26 Patzelt, V., in von Mollendorff, W. *Handbuch der mikroskopischen Anatomie des Menschen*, Berlin, Julius Springer, 1936, vol. 3.

tion and the associated drop of the gradient of pressure within the capillaries lead to an increase in the permeability of the capillary endothelium. As a consequence there is transudation of plasma into the tissue stroma, and if the process continues cellular components also pass through the capillary walls into the surrounding tissues. This is especially true of the red blood cells, and we thus obtain the punctiform hemorrhages already noted. These are considered, in the absence of any morphologic change in the vessel walls, to be the result of diapedesis. The process eventually terminates in necrosis of the tissue in the field of the blood supply of the affected vessels.

In this manner one may easily understand the occurrence of focal necroses in such a wide variety of clinical conditions.

#### SUMMARY

We have reported in detail 4 cases of ulcerative lesions in the esophagus, stomach and duodenum which appeared postoperatively or subsequent to diabetic acidosis. These are representative of the group of 47 cases which were reviewed in necropsy experience during a period of about ten years. In selecting these cases we omitted any in which a systemic disease might have caused the lesions, i. e., diffuse vascular disease, such as malignant sclerosis, chronic glomerulonephritis, periarteritis nodosa or sepsis. We also omitted cases in which an operative procedure had been performed on the stomach or in which a chronic ulcer of the stomach or duodenum was present.

A reconstruction of the sequence of histologic events on the basis of our observations indicates that the earliest change consists of marked distention in capillaries and venules, which is seen in the mucosa in the stomach and in the submucosa in the esophagus and duodenum. An anatomic explanation for this is offered. Edema fluid accumulates, and focal hemorrhages are frequently observed. These are due to the increase in permeability of the capillaries resulting from the stasis which is the consequence of the arteriolar constriction coming on in response to shock. The further progress of the process results in necrosis of the tissues in the foci involved.

#### CONCLUSION

From a study of a series of cases of acute ulcerative lesions of the esophagus, stomach and duodenum and from a review of similar cases in the literature, we have concluded that the vasomotor mechanisms which constitute the response to shock produce these lesions.

# ATROPHY OF THE CREMASTER MUSCLE

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The incidence of diseases which produce anatomic changes in striated skeletal muscle is relatively low. It was with this in mind that a search was made for a normal cremaster muscle to compare with a hypertrophied one. It was felt that it would be a simple matter to demonstrate a normal muscle. On the contrary, it was found necessary to examine many specimens before a cremaster muscle was found which had a normal gross and histologic appearance.

Three groups of specimens were examined in an attempt to determine the frequency as well as the possible cause of the changes that were found. The first group was picked at random from spermatic cords which had been removed together with the corresponding testes in the course of surgical repair of scrotal hernias. The second group was chosen from spermatic cords removed together with the corresponding testes in surgical treatment for testicular neoplasms. The third group consisted of spermatic cords obtained at necropsies.

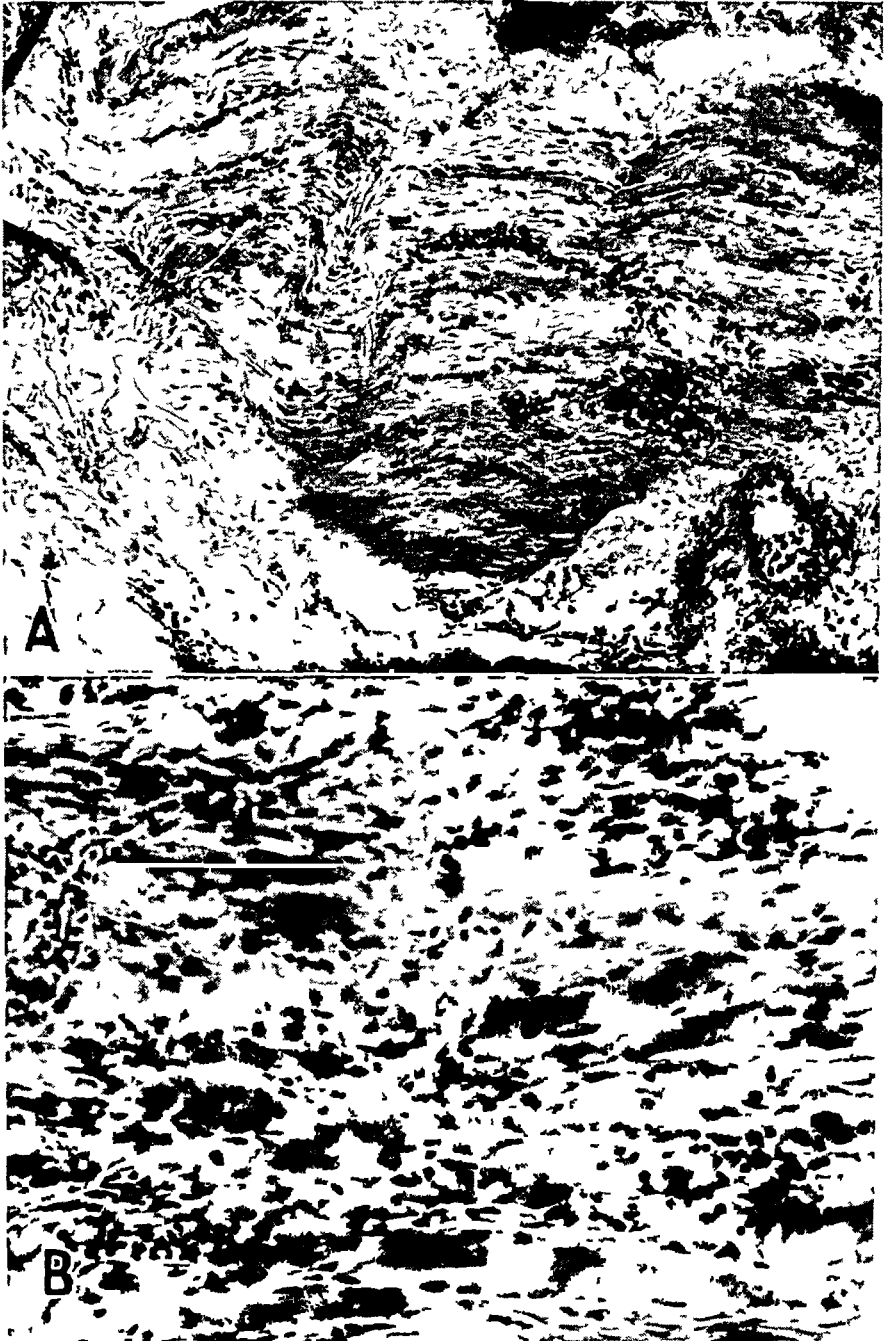
On each specimen a number of sections were made across the entire thickness of the spermatic cord at a distance of at least 2 cm above the epididymis. The sections were stained with hematoxylin and eosin. In addition, when the cremaster muscle could be identified grossly, longitudinal sections were made, mounted, and stained with hematoxylin and eosin. The sections were examined microscopically, and any variation from normal was noted.

In none of the spermatic cords examined was there hypertrophy. A total of 80 spermatic cords from different patients were examined. In no case were both cords of the same patient examined.

In 42 of the 80 spermatic cords it was impossible to demonstrate any cremaster muscle even after a careful search. Of the 38 cords in which striated muscle could be shown, the muscle was normal in only 9. In the remaining 29 specimens the muscle was infiltrated by varying numbers of lymphocytes. Occasionally polymorphonuclear leukocytes could be found scattered between the muscle fibers. In addition, a prominent change was the replacement of the muscle with collagenous

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*A*, cremaster muscle removed during the repair of a scrotal hernia, hematoxylin and eosin,  $\times 100$  There are multiple collections of lymphocytes with beginning fibrosis *B*, cremaster muscle showing marked fibrosis and many lymphocytes, hematoxylin and eosin,  $\times 220$

fibrous tissue (*A* in figure) In some cases this replacement was so marked that very few muscle fibers could be identified (*B* in figure)

Thirty-nine spermatic cords made up the first group examined, which were obtained from patients who had inguinal hernia A cremaster muscle could be identified in only a third of these specimens Of these, only a single muscle was normal The remaining 12 muscles showed infiltration by lymphocytes or fibrosis or both The patients from whom the spermatic cords in this group were obtained were older than those from whom the other two groups were obtained, many of them being over 50 years of age

The second group comprised 26 specimens representing cases in which the testis and a portion of the spermatic cord had been removed for a testicular neoplasm In half of these specimens it was possible to recognize a cremaster muscle Four of these muscles were normal on microscopic examination As a whole, the patients from whom this group were obtained were somewhat younger than those with inguinal hernia, from whom the first group were obtained

The third group comprised 15 specimens obtained at necropsies Striated muscle was present in all except 3 specimens of this group Four were normal The ages of the patients ranged from birth to 85 years

The average age of the patients from whom the specimens were obtained in which no cremaster muscle could be found was approximately 53 years The average age of the patients from whom the specimens were obtained in which cremaster muscle was identified but which showed fibrosis and lymphocytic infiltration was 51 years The average age of the patients from whom specimens showing normal cremaster muscle were obtained was 30 years

#### COMMENT

It appears that fibrosis of the cremaster muscle has an extensive distribution in human males It progresses with senescence and is seen less commonly in early life Probably it is a retrogressive phenomenon occurring in a relatively useless structure Trauma or incarceration of the spermatic cord undoubtedly plays a role, since fibrosis or absence of the cremaster muscle was very common in those cords that came from patients with inguinal hernia

Although the cremasteric reflex is absent in 26 per cent of newborn infants (Aronovitch<sup>1</sup>), it is present in all but 2.17 per cent of boys between the ages of 1 day and 10 years In boys the reflex may be so active that the testis rises with each inspiration and falls during expira-

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1 Aronovitch, G J Nerv & Ment Dis 64 235, 1926



tion (Jastrowsky <sup>2</sup>) Mitchell <sup>3</sup> and Purves-Stewart <sup>4</sup> observed that the cremasteric reflex is sluggish and sometimes absent in old men. Mitchell noted further that old hernias and trusses tend to lessen or destroy the cremasteric reflex. The absence or refractoriness of the cremasteric reflex in these old men can be explained entirely by the phenomenon of fibrosis and disappearance of the cremaster muscle. It is unlikely that changes in the reflex arc play an important role.

#### SUMMARY

The cremaster muscle frequently shows collections of lymphocytes with fibrous replacement of the muscle fibers. This change occurs most frequently in old men especially those with inguinal hernia. The absence or diminution of the cremasteric reflex in old men can be explained by these changes in the cremaster muscle.

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<sup>2</sup> Jastrowsky, cited by Mitchell <sup>3</sup>

<sup>3</sup> Mitchell, S. W. *J. Nerv. & Ment. Dis.* **4**: 577, 1879

<sup>4</sup> Purves-Stewart, J. *The Diagnosis of Nervous Diseases*, ed. 6, London, Edwin Arnold & Co., 1924

# NEW BONE FORMATION IN A PRIMARY CARCINOMA OF THE PROSTATE GLAND

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Carcinoma of the prostate gland with osteoplastic metastases to bone is a frequent and well known condition. However, bone in the stroma of a primary prostatic carcinoma has not previously been recorded. Nor has it been found in the soft tissue metastases of carcinoma of the prostate except for the case reported by Schmorl<sup>1</sup>. In this case bony-hard nodules were found in the lungs, and Schmorl interpreted the condition as osteochondrosarcoma developing in the stroma of the skeletal metastases of the prostatic carcinoma, with secondary sarcomatous and mixed deposits in the lungs. Heterotopic bone formation is not infrequent and occurs in many different organs and structures. However, it appears that bone formation in the prostate gland is extremely rare. Huggins<sup>2</sup> in listing the various extrasketal locations in which bone has been found did not include the prostate.

The case of prostatic carcinoma presented in this report is unique in that a nodule of bone measuring 10 by 14 mm was present deep in the prostate, intimately associated with the tumor cells. An additional feature of considerable interest was that in this nodule were a few foci of cartilage and one small area having the structure typical of the very rare gelatinous carcinoma of this organ.

## REPORT OF CASE

*Clinical History*—A white man aged 68 years was admitted to the United States Marine Hospital, Portland, Me., Dec 21, 1936, with the usual symptoms of prostatic carcinoma of about one year's duration. He had no pyuria, hematuria or recent loss of weight.

Physical examination disclosed a moderately enlarged hard prostate with areas of stony hardness in the left side. There was considerable reduction in the size of the prostatic urethra, a small metal catheter being passed with difficulty. The residual urine measured 8 ounces (236.5 cc), and excretion of phenolsulfonphthalein was retarded.

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From the Division of Pathology, National Institute of Health

1 Schmorl. Verhandl d deutsch path Gesellsch **12** 89, 1908

2 Huggins, C B. Arch Surg **22** 377, 1931

A suprapubic cystotomy was done December 26, followed by prostatectomy Jan 15, 1937. The large indurated prostate was enucleated with difficulty and showed two quite hard, apparently calcified areas.

Examination June 15 showed a loss of 8 pounds (3.6 Kg) in weight. The patient was almost totally incontinent. Urinalysis showed a slight trace of albumin and few white blood cells. There was a very large hard recurrent prostatic tumor, and nodular masses extended up both sides of the pelvis, obliterating all landmarks. Treatment with roentgen rays of high voltage was begun on June 16 and continued until November 10. A total of 7,300 roentgens was given. Jan 31, 1938, by letter, the patient reported his condition as unimproved. No further information has been obtainable up to the time of writing (December 1938).

This history was furnished by Dr. J. E. Hammond and Dr. M. B. Noyes of the United States Marine Hospital, Portland, Me.

*Microscopic Examination of Prostate*—In each of two blocks there was a well circumscribed nodule showing increase in the number and size of alveoli, lined by hyperplastic and focally stratified columnar epithelium. In some areas there were fairly prominent intra-alveolar papillary projections. Marginating these nodules and in three other blocks there was moderate to marked invasion of the stroma by variably sized tubular acini, alveoli, atypical tubules and solid or multiluminate sheets of cells. Some of the alveoli showed many papillary ingrowths, the fusion of which gave a fenestrated appearance. The cells were cubic, columnar or polygonal and had ample clear or lightly oxyphilic cytoplasm and large round hyperchromatic vesicular or leptochromatic nuclei, usually without distinct nucleoli. Mitoses were infrequent. There was marked tumor invasion of the capsule and of the muscular wall of the seminal vesicles. Here the acini were generally small and numerous and were seen as deep as the base of the mucosal folds. Tumor cell groups were seen in a few lymphatics, particularly the perineural vessels.

The grossly stony nodule measured 10 by 14 mm and was formed largely of masses and ramifying trabeculae of bone, apparently of the membranous type. The spaces between the bone lamellae frequently contained large masses of tumor cells or a few loosely disposed tumor cells set in a fibrous stroma. Occasionally these tumor cells were present just within the bone lamellae. A layer of dense fibrous tissue separated the bony nodule, at least in part, from the remainder of the gland. Tumor acini were present only in the outer margin of the nodule. There was one poorly circumscribed, irregularly shaped area, measuring 2 by 3 mm, in which tumor cells were arranged in columns or strands separated by bands of fibrillary mucin (stained red with mucicarmine and metachromatic with toluidine blue). The bands were frequently from two to three times as thick as the cell columns. This structure graded on one margin into solid tumor tissue, on the opposite side mucin was more copious and single cells were isolated. The latter margin abutted directly on atypical cartilage having large lacunae and much thready metachromatic matrix. A few cell-containing lacunae surrounded by a moderate amount of matrix occurred singly or in small groups in marginating bone. The tumor cells in this nodule were large and irregular in shape, and had quite hyperchromatic nuclei and distinct nucleoli. Few multinucleate cells were seen. Mitoses were moderately numerous, two of which occurred in cells within lacunae.

A second area, measuring 2 by 3 mm, showed larger and more numerous lacunae surrounded by a thready metachromatic matrix in which a few collagen fibrils occurred. The collagen fibrils were more numerous in the periphery of the nodule and were continuous with the surrounding fibrous tissue.

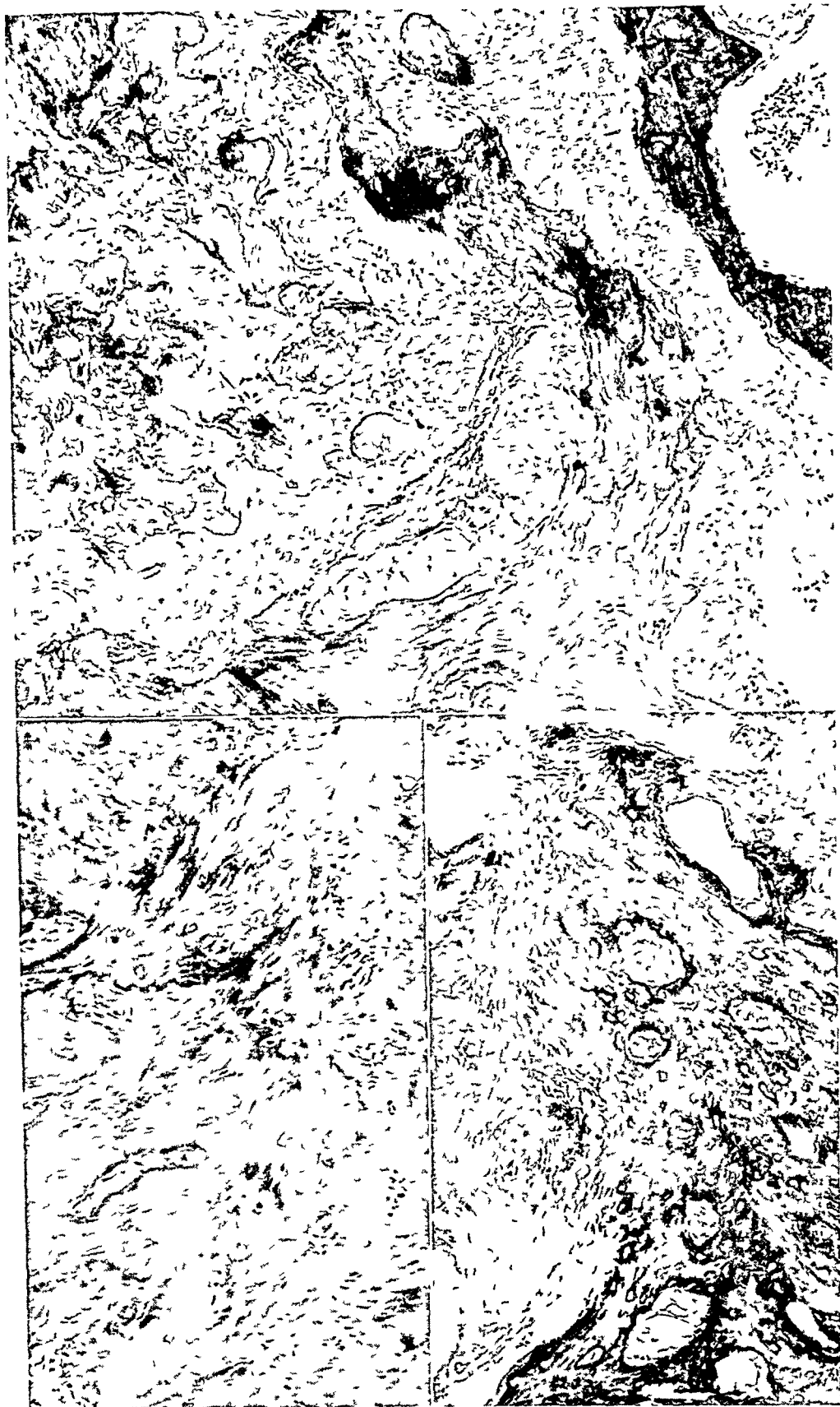


Fig 1—*A*, lamellae of bone separated by sheets of tumor cells Van Gieson's stain,  $\times 80$  *B*, irregularly directed bone lamellae separated by fibrous tissue in which occasional to few tumor cells occur Van Gieson's stain,  $\times 80$  *C*, section showing gradation from bone to fibrous tissue Van Gieson's stain,  $\times 80$

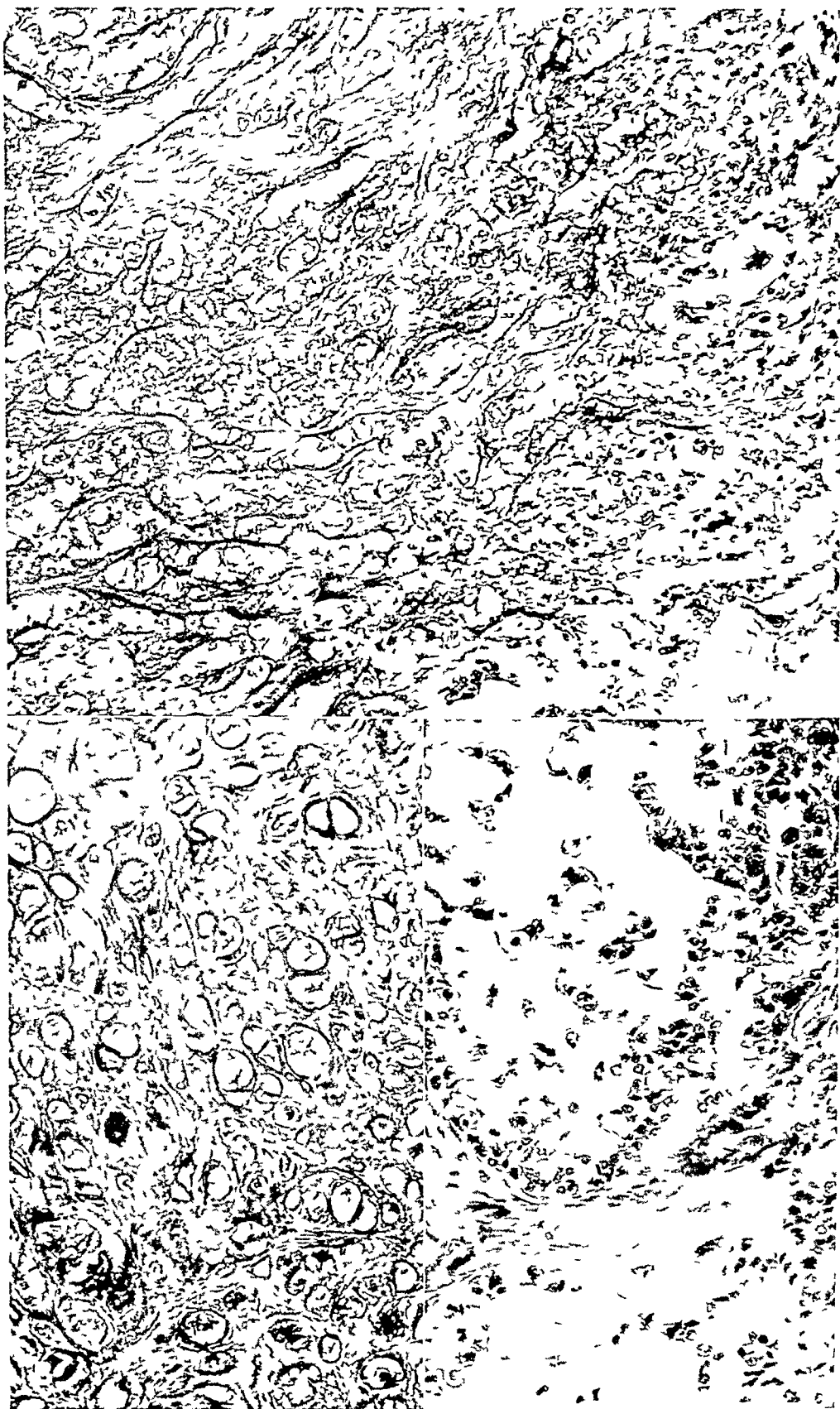


Fig 2—*A*, solid tumor at right, much intercellular thready mucin at left. Note the intergradation. Toluidine blue stain,  $\times 130$ . *B*, area of atypical cartilage, the matrix is thready and metachromatic. Toluidine blue stain,  $\times 130$ . *C*, area having the structure of gelatinous carcinoma (similar to *A*). The intercellular thready mucin is not stained. Van Gieson's stain,  $\times 130$ .

## COMMENT

The mechanism of bone formation in the skeletal metastases from carcinoma of the prostate has been the subject of much discussion, and numerous theories have been formulated. Von Recklinghausen<sup>3</sup> in 1891 gave a classic description of this condition and suggested that local congestion resulting from numerous tumor thrombi was the factor initiating the bone growth. Courvoisier<sup>4</sup> thought that the osteogenesis resulted from the accompanying inflammatory reaction. Assmann<sup>5</sup> believed that primary necrosis of bone was necessary before new bone could be formed. Quite contrary to these views was the suggestion by Axhausen<sup>6</sup> that the cancer cells liberated a chemical factor or stimulus which initiated the formation of bone. He did not believe that the cancer cells assumed the function of osteoblasts, a belief held by Braun,<sup>7</sup> Kaufmann<sup>8</sup> and Sasse.<sup>9</sup>

Recent studies on this subject have borne out the view held by Axhausen. Gutman, Sproul and Gutman<sup>10</sup> showed that there is increased phosphatase activity of bone at the site of osteoplastic metastases secondary to carcinoma of the prostate gland. They feel that the capacity of the tumor cells to stimulate production of bone phosphatase determines in part the osteoplastic character of the metastatic lesion. Huggins<sup>11</sup> showed that in dogs bladder epithelium transplanted to the sheath of the rectus abdominis muscle has the property of initiating formation of bone and that there is increased phosphatase activity<sup>12</sup> in these heterotopic foci of bone.

Since prostatic epithelium is closely related embryologically to bladder epithelium, it may be true, as suggested by Huggins,<sup>2</sup> that prostatic epithelium also has "osteogenic" properties. This assumption would serve as an attractive theory in explaining the presence of bone in the case presented here. The bone was of the membranous type, apparently being formed directly from dense fibrous tissue, and although a few

3 von Recklinghausen, F. Die fibrose oder deformierende ostitis, die Osteomalacie und die osteoplastische Carcinose in ihren gegenseitigen Beziehungen, in Arbeiten aus dem Kaiser- und Kaiserin- Friedrich- Kinderkrankenhaus in Berlin, Festschrift, Herrn Rudolph Virchow zum 70. Geburtstage, Stuttgart, Ferdinand Enke, 1891, pp. 1-89.

4 Courvoisier, W. Das Prostatacarcinom, Inaug. Dissert., Basel, M. Werner-Riehrn, 1901.

5 Assmann, H. Virchows Arch. f. path. Anat. **188**, 32, 1907.

6 Axhausen, G. Virchows Arch. f. path. Anat. **195**, 358, 1909.

7 Braun, L. Wien med. Wchnschr. **46**, 481, 527 and 582, 1896.

8 Kaufmann, E. Lehrbuch der speciellen pathologischen Anatomie für Studierende und Aertze, Berlin, George Reimer, 1911, pp. 759-762.

9 Sasse, F. Arch. f. klin. Chir. **48**, 593, 1894.

10 Gutman, E. B., Sproul, E. E., and Gutman, A. B. Am. J. Cancer **28**, 485, 1936.

11 Huggins, C. B. Proc. Soc. Exper. Biol. & Med. **27**, 349, 1930.

12 Huggins, C. B. Biochem. J. **25**, 728, 1931.

isolated cancer cells were seen in the peripheral margins of some bone lamellae, they did not appear to be assuming the function of osteoblasts. Bone cells in all lamellae were of the adult, small type. Osteoblasts palisaded against bone were not found. Since this case shows that cells capable of acting as osteoblasts are present in the prostate, the question naturally arises: Why is bone not found more often in cancerous prostates? This question cannot at present be answered. However, Huggins, McCarroll and Blocksom<sup>13</sup> found that the corium of the urinary bladder has an inhibiting influence on the osteogenic property of the overlying epithelium and that this inhibiting factor is not present in the outer part of the bladder wall. Huggins<sup>2</sup> saw no production of bone in transplants of prostatic tissue. He suggested that the intimately associated stroma, which could not be removed from the epithelium, might have an inhibiting influence similar to that of bladder mucosal corium. If the prostatic stroma does have an inhibiting influence on bone formation, this case demonstrates that it can be overcome under some conditions.

The areas in the hard nodule having the structure of gelatinous carcinoma are interesting because of the rarity of this type of carcinoma of the prostate. Boyd<sup>14</sup> reported such a case, and more recently Klissurow<sup>15</sup> made a detailed study of a case in which carcinoma of the prostate gland of the usual type was present except in the left lobe. In this lobe it was of the gelatinous type. Considering the embryology of the gland and the fact that Pilcher<sup>16</sup> demonstrated the presence of variable amounts of mucus in a large percentage of cases of carcinoma of the prostate gland, it is surprising that more cases of frank gelatinous carcinoma are not found.

The relationship of the area of mucous carcinoma to the adjacent cartilage is interesting but difficult to understand. For the greater part, there was abrupt change from one type of tissue to the other. However, at one point there appeared to be a transition, and the cells within the lacunae appeared quite similar to the cancer cells lying between the strands of mucus. Also, a few of the cartilage cells were seen in mitosis.

#### SUMMARY

A case of carcinoma of the prostate gland is presented in which a stony-hard nodule was present composed mainly of bone but showing also foci of cartilage and one area having the structure of gelatinous carcinoma. The possible significance of the bone formation is discussed.

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13 Huggins, C. B., McCarroll, H. R., and Blocksom, B. H., Jr. *Arch. Surg.* **32**: 915, 1936.

14 Boyd, S. *Tr. Path. Soc. London* **33**: 200, 1882.

15 Klissurow, A. *Virchows Arch. f. path. Anat.* **268**: 515, 1928.

16 Pilcher, F., Jr. *Am. J. Clin. Path.* **8**: 366, 1938.

# DISSEMINATED ENCEPHALOMYELITIS OF THE DOG

LESTER S KING, M D

PRINCETON, N J

In 1930 Perdrau and Pugh,<sup>1</sup> in England, called attention to demyelinating lesions in the central nervous system of the dog suffering from what they called "the 'nervous form of canine distemper'" They found that 4 of 14 animals showed this reaction The demyelination, when present, was observed chiefly in the cerebellar peduncles or folia and was generally accompanied by heavily cuffed blood vessels

At the same time, but independently, Posrednik<sup>2</sup> described similar lesions In 9 cases of experimentally induced distemper with a greater or a less degree of encephalitis, he observed a very slight degree of demyelination in 1 instance But in 3 cases of the disease occurring naturally the loss of myelin was more pronounced, especially in the cerebellum, medulla and pons Subsequently Marinesco, Draganesco and Stroesco,<sup>3</sup> as well as Peters and Yamagiwa,<sup>4</sup> gave detailed descriptions of the histologic changes in cases of distemper and confirmed the observation of loss of myelin in many of the lesions

The literature on distemper is extensive This virus-induced disease may attack the nervous system to produce nervous manifestations but does so inconstantly Apart from the loss of myelin which is sometimes observed, the principal features described by the older investigators are those of encephalitis and meningoencephalitis, namely perivascular accumulation of lymphocytes, inflammatory infiltrations involving the parenchyma and the meninges, sometimes very intensive, occasional perivascular hemorrhage, endothelial proliferation, variable degrees of degenerative neuronal damage, changes in the glial apparatus, both proliferative and regressive The intracellular inclusions, first described thirty years ago, have recently been studied and the relevant literature reviewed by De Monbreun<sup>5</sup> He found the inclusions abundantly present

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From the Department of Animal and Plant Pathology of the Rockefeller Institute for Medical Research

1 Perdrau, J R, and Pugh, L P J Path & Bact **33** 79, 1930

2 Posrednik, F I Ztschr f Infektionskr **38** 135, 1930

3 Marinesco, G, Draganesco, S, and Stroesco, G Ann Inst Pasteur **51** 215, 1933

4 Peters, G, and Yamagiwa, S Arch f Tierh **70** 138, 1935

5 De Monbreun, W A Am J Path **13** 187, 1937



in the brain as well as in other diseased tissues. Many authors, however, have not been able to find inclusions in their cases, although, among recent writers on the neuropathology of the disease, excellent illustrations have been given by Gallego<sup>6</sup> and by Mainesco, Draganesco and Strosco,<sup>3</sup> as well as by De Monbreun.<sup>5</sup>

The observations mentioned are those which occur when the animal shows clinically involvement of the nervous system. But Cerletti<sup>7</sup> described a series of dogs suffering from clinical distemper without nervous manifestations in which, nevertheless, inflammatory and toxidegenerative lesions of the nervous system were present. This observation has been confirmed by Roman and Lapp.<sup>8</sup>

Although the foreign literature contains numerous studies on disseminated encephalomyelitis of the dog and its relation to distemper, little attention has been paid to the subject in this country. No observations have been made on the problem of demyelination. The following report of a case is consequently of some neuropathologic interest.

#### CLINICAL HISTORY

The dog reported on here was sent to this laboratory by Dr. F. A. Zucker, of Roselle, N. J.

The available history is rather sketchy. The animal was a pointer bitch, about 4 years old, first taken ill about three months previously. The original symptoms are not on record, but the animal was first treated by a veterinarian for tapeworm. A second veterinarian diagnosed brain fever. When seen by Dr. Zucker, the animal showed spasticity, stiffness of gait, with clonic movements of the forelegs, ataxia and mental impairment. The pupils did not react to light, and there was no response to pinprick over the posterior portion of the body. There was slight improvement for a few days, but then the animal became rapidly worse and was sent to this laboratory for examination.

When seen by me, the animal was in a recumbent position, lying on the right side, in marked distress. There were marked decubitus ulcers on the right shoulder and elbow. The hindlegs were flexed and completely paralyzed. Moderate contractures were present. The right foreleg was in rigid extension and strongly resistant to any passive movement. The left foreleg was only moderately spastic. No tendon reflexes were elicitable. The sensory deficit was marked, although no sharp level could be detected. Pinching and mildly painful stimuli applied to the hindlegs and trunk evoked no response. Some sensation apparently remained in the forelegs and head. Pupillary and corneal reflexes were present.

The animal was killed by chloroform anesthesia. During the struggle only the left foreleg and the head were spontaneously moved. During narcosis the spastic right foreleg became flaccid. The hindlegs showed only moderate relaxation due to the contractures.

The general autopsy showed only severe hemorrhagic cystitis. The bladder was not dilated, but the wall was markedly thickened, and the mucosa showed

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6 Gallego, A. *Ztschr. f. Infektionskr.* **34** 38, 1928.

7 Cerletti, U. *Ztschr. f. d. ges. Neurol. u. Psychiat.* **9** 520, 1912.

8 Roman, B., and Lapp, C. M. *Bull. Buffalo Gen. Hosp.* **3** 40, 1925.

inflammation, hemorrhage and necrosis. There were no other features of significance. The involvement of the bladder was undoubtedly secondary to the injury of the spinal cord.

With regard to the nervous system, in gross the most marked changes were in the spinal cord, which in portions was very soft. On section cavities were visible in some regions around the central canal. The brain showed no gross abnormalities.

The brain and cord were fixed in 10 per cent formaldehyde, pyroxylin, paraffin and frozen sections were made of representative regions.<sup>8a</sup> Hematoxylin-eosin, thionine, iron-hematoxylin, Van Gieson, scarlet red, Weil, silver carbonate, Bodian<sup>8b</sup> and Anderson<sup>8c</sup> staining methods were used.

#### MICROSCOPIC OBSERVATIONS

The changes in the nervous system are primarily inflammatory, although varying in intensity. The diseased areas are scattered throughout the entire neuraxis, with the greatest intensity in the spinal cord. The lesions in the cord are not confluent but scattered throughout its length. Some lesions, however, extend over several segments.

*Spinal Cord*—A typical lesion is a fairly well delimited focus occurring in either the gray or the white substance but with greater frequency in the latter. Figure 1 *A* illustrates such a lesion. The blood vessels stand out because of the perivascular infiltration by lymphocytes, plasma cells and more undifferentiated mononuclear cells. Under higher power the endothelium may, in places, be seen to be proliferated. Between and around the blood vessels the tissue is rarefied, presenting a lacunar appearance, but axis-cylinders persist in large numbers. Apart from the sheaths of blood vessels there is little or no increase in cellularity in the damaged area. Although some progressively altered glial forms may be observed, there are no gitter cells in this particular lesion.

There are several variations on this picture each of which presents its own significant features. Two fields from a single section illustrate different pathologic processes. In figure 1 *B* there is shown a very intense cellular reaction. The larger blood vessels (outside the field of this photomicrograph) are very heavily cuffed, but in the particular field shown the infiltration of the tissue by plasma cells is noteworthy. Gitter cells are present in moderate numbers, as well as other microglial forms. Occasional polymorphonuclear leukocytes are also scattered through the tissue. The lacunae in the tissue may, to a certain extent, be seen in process of formation, but the cellular infiltration is so heavy that details of the tissue are obscured. This is considered to be an

8a The Weil staining method is described in McClung, C. E. *Handbook of Microscopical Technique*, New York, Paul B. Hoeber, Inc., 1937, pp. 474.

8b Bodian, D. *Anat. Rec.* **65**: 89, 1936.

8c Anderson, J. *How to Stain the Nervous System*, Edinburgh, E. & S. Livingstone, 1929, p. 80.

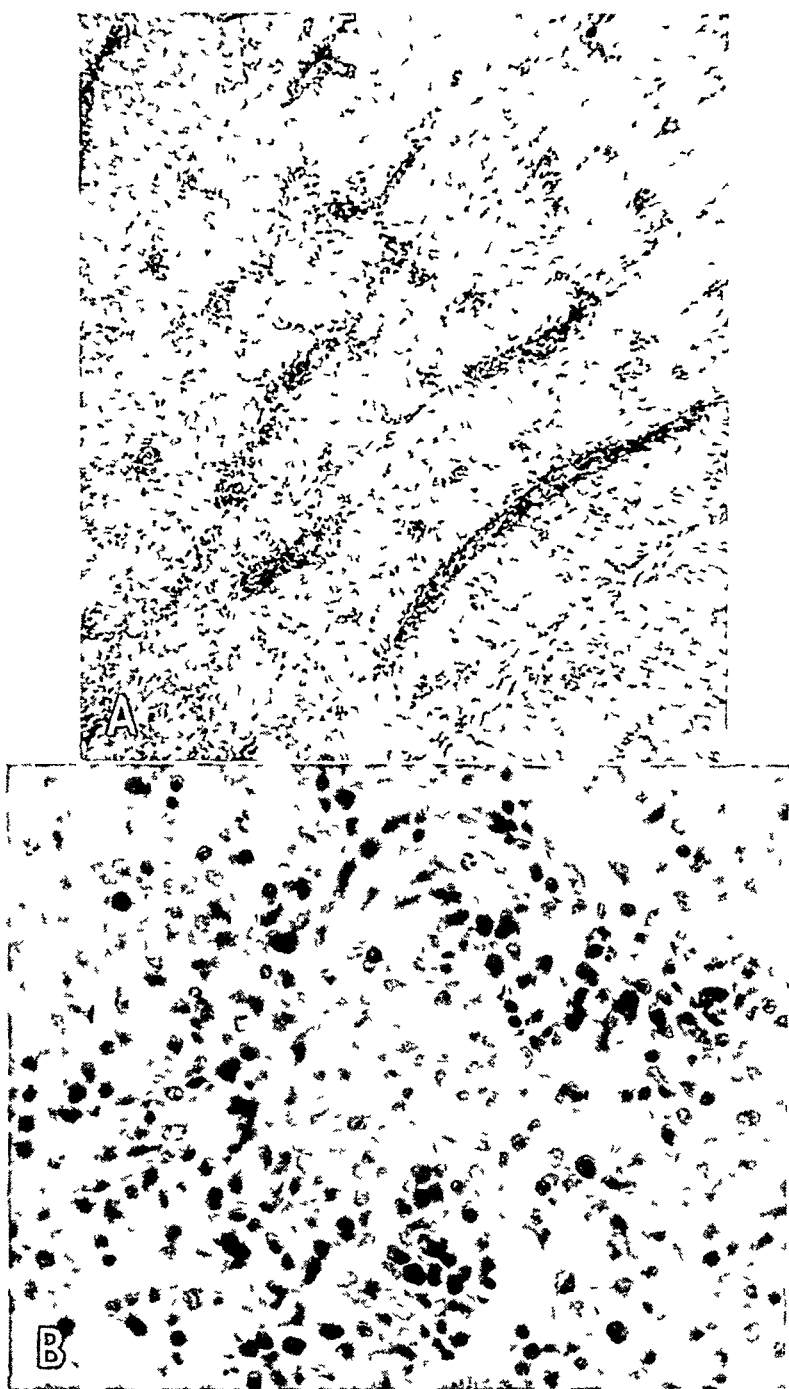


Fig 1—*A*, spinal cord, anterior-lateral portion. There are moderately intense perivascular infiltration and proliferation of vascular endothelium. The cribrated appearance of the white matter should be noted. On the extreme lower right, the myelin is essentially normal. Hematoxylin-eosin stain,  $\times 615$ . *B*, spinal cord, showing infiltration of the parenchyma with plasma cells and occasional polymorphonuclear leukocytes. Hematoxylin-eosin stain,  $\times 4312$ .

acute stage of the process shown in figure 1 *A*. On the other hand, elsewhere the *état criblé* may be very marked, even more than in figure 1 *A*, and yet the interstitial reactions may be entirely negligible.

A quite different picture is shown in figure 2 *A*. Here there is practically complete softening, involving the anterior horn and adjacent white matter. The reactive cells are almost exclusively compound granular corpuscles, and these are not numerous. Most unusual, however, is the excellent state of preservation of the anterior horn cells in the midst of the tissue debris. The nuclear membranes are hyperchromatic, but the general cellular appearance is excellent. There are a few neurons that show degenerative changes, but they are exceptional. However, in other areas, in other sections of the cord, a similar type of damage of the tissue has destroyed the neurons as well.

Sections of the spinal cord stained for myelin show expected changes. Figure 2 *B*, of the cervical region, displays different degrees of demyelination, in accordance with the cellular picture. The regions of spongy vacuolation and rarefaction, with moderate perivascular reaction, show considerable diminution in the quantity of myelin but not total loss. Other areas show practically complete destruction of myelin. In the center there is cavitation, due to complete softening. Many of the gutter cells contain hematoxylin-staining masses of myelin, testifying to the acuteness of the process. Longitudinal sections of the cord (fig 2 *C*) show an irregular margin as well as the usual beadings and swellings attendant on myelin disintegration.

An instructive field is illustrated in figure 2 *D*, taken from the lateral column of a cross section of the thoracic portion of the cord. Numerous punched-out areas of demyelination are present in otherwise well preserved tissue. Closer examination shows that all of these areas are heavily cuffed blood vessels, where the perivascular accumulation of cells has choked out the myelin. Some of the inflammatory cells contain minute droplets of myelin. The intervening tissue appears relatively normal. This type of demyelination seems to be in a different category from that previously illustrated. It is more comparable to the strictly focal demyelination sometimes seen after hemorrhagic encephalitis<sup>9</sup> rather than to the diffuse damage of tissue seen in figure 1 and figures 2 *A*, *B* and *C*. It is very doubtful that this perivascular type would develop into the diffuse forms, in which the tissue as a whole appears injured. The pathogenic factors in the two forms appear to be distinct.

Sections of the spinal cord stained for fat confirm the observations on the sections stained with hematoxylin and eosin. The amount of neutral fat, although in some areas fairly considerable, is on the whole much

<sup>9</sup> Baker, A. B. *Am J Path* **11** 185, 1935. Russell, D. S. *J Path & Bact* **45** 357, 1937. Dobbs, R. H., and de Saram, G. S. W. *ibid* **46** 437, 1938.

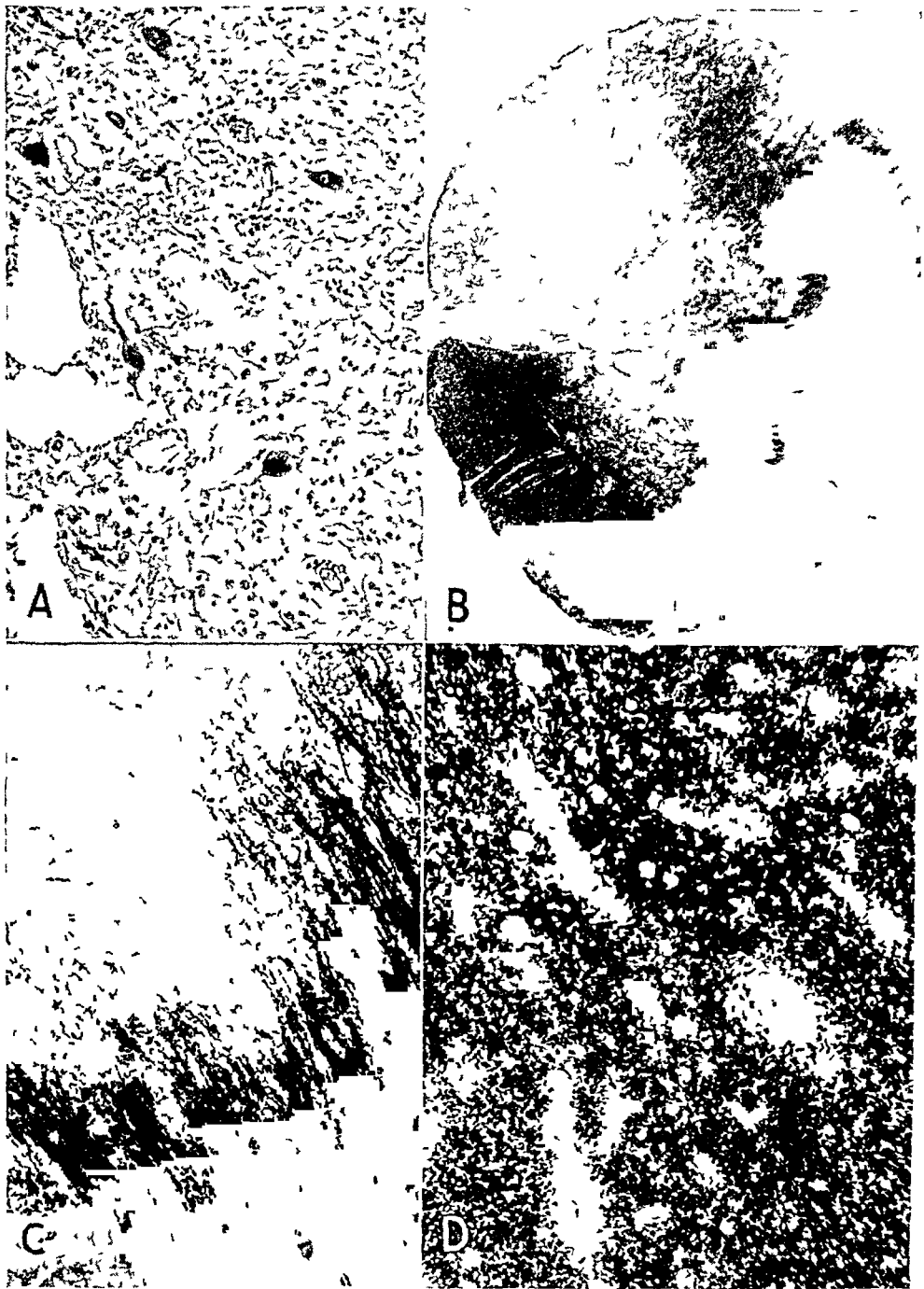


Fig 2—*A*, spinal cord. There is almost complete softening of part of the anterior horn and adjacent white matter. The reactive cells are almost exclusively compound granular corpuscles. The excellent preservation of the ganglion cells in the midst of the tissue debris should be noted. Hematoxylin-eosin stain,  $\times 85.8$ . *B*, spinal cord, showing partial and complete demyelination. The etat crible is readily apparent. Weil stain,  $\times 94$ . *C*, spinal cord, longitudinal section, illustrating the sharp margin of the demyelinating process. Much hematoxylin-staining material is still present in the demyelinated area. Weil stain,  $\times 34.5$ . *D*, transverse section of spinal cord, showing perivascular demyelination consequent to intense perivascular infiltration. There is much phagocytosed myelin in the perivascular spaces. Weil stain,  $\times 72.7$ .

less than would be expected, certainly incomparably less than would be seen in so-called acute multiple sclerosis of comparable duration. The gitter cells, as has already been noticed, are not very numerous except in certain areas of almost total softening. The Herxheimer stain shows many cells, both in the perivascular sheaths and free in the tissue, which contain a few small globules of neutral fat. The heavily crammed compound granular corpuscles are present in certain areas, but where the infiltration of the tissue is heaviest they are few.

The axis-cylinders are invariably better preserved than the myelin. Even where the destruction of tissue appears most complete, axis-cylinders persist, though distorted and damaged. In other areas they are preserved in considerable numbers. All types of degenerative changes, such as varicosities, torpedoes, swellings, splittings, fibrillary ball formations, loops and the like, are abundantly in evidence.

Repair has taken place to a certain extent, but instead of gliosis (i. e., proliferation of fibrous astrocytes and their fibers) chiefly the connective tissue is involved. Figure 3 *A* illustrates a rather marked degree of this process. For comparison with the connective tissue reaction in multiple and diffuse sclerosis, reference may be made to a previous paper.<sup>10</sup>

*Medulla and Higher Levels*—The pathologic changes differ from those of the preceding description chiefly in degree. In general they are not so severe as those in the spinal cord.

In the medulla and pons there are scattered lesions involving the cerebellar peduncles, various scattered areas at the periphery and less frequent areas in the more central portions. Figure 3 *B* illustrates the degree of damage in the pons. In general there is fairly heavy perivascular cuffing with plasma cells and lymphocytes. Rarefaction of tissue and lacunar degeneration are common, but white blood cells are not frequent in the parenchyma. Numerous compound granular corpuscles are present. Apart from the discrete lesions the tissue is entirely normal.

In the cerebellum too the process is similar. The damaged areas are fairly well circumscribed and scattered through the cerebellar white matter. The perivascular infiltration is often quite dense, and infiltration of the tissue by inflammatory cells may be well marked. However, it is nowhere as severe as in the spinal cord. In the cerebellum itself, in contrast to the cerebellar peduncles, medulla and cord, there is much less of the *état criblé*. The demyelination is more complete and not attended by lacunar degeneration of the tissue. Figure 3 *C* illustrates lesions in the cerebellar white matter.

Areas may be seen similar to those in figure 2 *D*, i. e., small "holes" in the myelin caused by the heavily cuffed blood vessels. The intervening

<sup>10</sup> King, L. S. Arch Path 23 338, 1937



Fig 3—*A*, reticulin proliferation in a diseased area of the spinal cord. Silver carbonate impregnation for connective tissue,  $\times 716$ . *B*, brain stem cut through the inferior colliculi and pons, showing demyelination in the pons. Arrows point to less obvious areas. Weil stain,  $\times 36$ . *C*, demyelination in the cerebellum. Arrows point to early foci. Weil stain,  $\times 425$ . *D*, inflammatory focus in the caudate nucleus, bordering the ventricle. Foci in the cerebral cortex are very similar. Hematoxylin-Van Gieson stain,  $\times 305$ .



Fig 4—*A*, cerebral cortex illustrating the loss of radial and tangential fibers in a cortical area of inflammation. Weil stain,  $\times 184$ . *B*, tissue showing experimental canine distemper (from a preparation supplied by Dr De Monbreun). The tissue is from the region of the dentate nucleus. The similarities to the picture described for the dog reported here are discussed in the text. The arrow points to a neuron with a prominent intranuclear inclusion. Hematoxylin-eosin stain,  $\times 165$ .



myelin may at times appear lighter and less dense than in perfectly normal areas (In the illustration, the partial involvement of the roof nuclei, naturally lighter than the white matter, should not cause confusion) These "punched-out" areas usually appear, as in the spinal cord, at the margin or near much more severe damage

The axis-cylinders may show moderate diminution in number and are nowhere as well preserved as they may be in some instances of multiple sclerosis But the destructive action on the axis-cylinders is not so severe in the cerebellum as in the spinal cord The usual degenerative alterations may occasionally be found

There is considerably more gliosis, generally isomorphous in type, in the cerebellar lesions than in the spinal cord Monster astrocytes are also present The connective tissue proliferation and the reticulin nets, although present, are much less exuberant than in the spinal cord

Occasionally in the cerebellar cortex there is an out-dropping of Purkinje cells, without vascular reaction This change, fairly common in many pathologic conditions is never severe

Lesions such as have been described are also present in the white matter elsewhere in the brain—for example, in the optic chiasm and tracts and in the hippocampal commissure But no damage has been detected in the corpus callosum or the centrum ovale, where Marinesco, Draganesco and Stroesco<sup>3</sup> described demyelination

Scattered in the basal ganglions and cerebral cortex are numerous circumscribed lesions The usual inflammatory changes are present around the blood vessels, and in the parenchyma there is some glial mobilization, as well as invasion in places by mononuclear cells The nerve cells are in general excellently preserved, although there may be some increase in satellitosis Figure 3 *D*, from the ventricular surface of the caudate nucleus, is typical of the lesions found both here and in the cerebral cortex There is little true destruction of tissue, as found in the spinal cord Appropriate stains, however, show loss of myelin Figure 4 *A*, from the neocortex, shows loss of radial and tangential fibers at the site of inflammation It will be observed that the underlying white matter is entirely normal It is noteworthy that in these foci in the cerebral gray matter there is intense proliferation of fibrous astrocytes

Careful search was made for intranuclear inclusions at all levels, but none was found

#### COMMENT

This case is pathologically similar to those described by Perdrau and Pugh<sup>1</sup> and by Marinesco, Draganesco and Stroesco<sup>3</sup>, in all the condition may properly be called "disseminated encephalomyelitis with demyelination" The relation to distemper is not clear Perdrau and

Pugh showed that this form of encephalomyelitis may occur long after, or even in the complete absence of, clinically recognizable distemper.

On the other hand, De Monbreun, studying distemper experimentally, produced and described lesions in dogs which appear to be similar to the demyelination described by others. Dr. De Monbreun sent me a preparation, a field from which is reproduced, with his permission, in figure 4 *B*. The field is from the region of the dentate nucleus of the cerebellum. The extensive damage of tissue, with numerous gutter cells, the inflammatory reaction around the blood vessels and the invasion of the parenchyma by inflammatory cells are clearly in evidence. (The neuron designated by the arrows shows a brilliantly acidophilic intranuclear inclusion, which cannot be well appreciated however in the photograph.) Such a lesion stained for myelin would show demyelination.

It is well established that in distemper as well as in other diseases the inflammatory changes in the nervous system are not of themselves sufficient to induce loss of myelin. The demyelination, according to Perdrau and Pugh,<sup>1</sup> "is not the result of the action of the specific virus of distemper on the central nervous system," but "the virus of distemper plays in this disease of the dog a similar role to that which an acute infection of varying etiology plays in the causation of certain demyelinating diseases of man."

From the work of previously cited authors the conclusion might be drawn that demyelinating lesions may appear in the course of distemper, but also as an indirect, temporally removed consequence, as well as independently of this disease.

Certain features of the case reported here deserve comment. Different types of myelin loss may be seen. First, there is that which is attendant on practically complete destruction of tissue with inflammatory reaction (figs. 1 *B* and 2 *A*). Second, there is the strictly perivascular damage of myelin, appearing around heavily infiltrated blood vessels, where the distended perivascular sheaths have encroached on the parenchyma (figure 3 *A*). Third, there is the more diffuse and often incomplete loss of myelin, sometimes with *état criblé*. In the latter type there may be no inflammatory change in the parenchyma or varying degrees of change up to a very moderate intensity.

In considering the problem of demyelination it is necessary to distinguish sharply between destruction of tissue as a whole and more selective destruction of myelin. Various forms of encephalitis caused by viruses may produce a generally destructive process, in which all tissue elements are severely damaged. There is nothing selective about the process, and the myelin disappears only as a part of the general tissue disintegration. In animals this has been described especially in equine

encephalomyelitis<sup>11</sup> There are many instances of myelitis and encephalomyelitis in man in which the observations are similar

Opposed to the general damage of tissue, in which myelin suffers along with other tissue elements, there may occur in some diseases a selective destruction of myelin In some instances of multiple sclerosis in which there is a sharp border of demyelination, the axis-cylinders within the lesion are essentially intact Although gutter cells may be present in large numbers, there are generally no "inflammatory" cells Such a picture, common in multiple sclerosis, was present to some extent in certain areas of the dog described here, especially in the cerebellar lesions This selective attack on myelin is quite different from the general destruction of tissue found in many diseases proved to have viruses as their etiologic factors as well as in many of unknown cause

A still different type of demyelination in the dog reported here is the *état crible*, visible in figures 1 *A* and 2 *B* This mode of reaction is quite different from multiple or diffuse sclerosis but is comparable to what is found in combined system disease The problem of a dietary deficiency superimposed on the original disease process in this dog must remain open

There are certain similarities to many of the reported cases of neuromyelitis optica, namely, marked involvement of the optic tract and of the spinal cord, with scattered lesions elsewhere Very intense inflammation with widespread destruction of tissue is a feature of many instances of Devic's disease It is only the latter cases that resemble the one reported here

There has been much discussion in the literature regarding the possible nosologic unity of the different demyelinating diseases To me it seems clear that the disease described here, together with most of the disseminated encephalomyelitides with demyelination recorded in the literature, belongs in a totally different category from multiple sclerosis Neuromyelitis optica belongs in or very near the former group Diffuse sclerosis is not a disease entity, but certain of its subdivisions may be related to the first group, others to the second, still others may stand sui generis These views are a matter of opinion only, as are other views expressed in the literature, and must remain so until further etiologic data are available

#### SUMMARY

Disseminated encephalomyelitis with demyelination in a dog, of about three months' duration, is presented There were intense inflammatory changes, with very severe involvement of the spinal cord, and scattered lesions elsewhere including the optic chiasm and tracts Different types of demyelination and the accompanying pathologic features are described

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11 King, L S J Exper Med 68 677, 1938

# PRODUCTION OF XANTHOMA IN RABBITS

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It is generally believed that xanthoma is in some way associated with a disturbance in lipid metabolism, but relatively little experimental work on the production of xanthoma has been reported. In rabbits fed cholesterol<sup>1</sup> lipid deposits have been found in the sclera, the cornea, the ciliary body of the eye, the tympanic membrane and tendons. Anitschkow<sup>2</sup> produced typical xanthoma in rabbits by feeding cholesterol and at the same time traumatizing certain areas by injections of colloidin or turpentine. Since xanthoma nodules occurred at the injured sites, he concluded that two factors were necessary for their production—cholesteremia and trauma. Schaaf<sup>3</sup> produced xanthoma in 9 rabbits by feeding hydrous wool fat for periods up to seven hundred days. All of his animals were traumatized mechanically by a clamp on the neck and chemically by intradermal injections of lipid mixtures. Furthermore, most of the animals suffered from a derangement of the liver due to action of roentgen rays, arsphenamine or para toluenediamine. Xanthoma appeared in the traumatized areas, but changes occurred also in other parts of the body, and the various complicating procedures failed to produce xanthoma in the absence of cholesteremia.

In connection with experiments on ultraviolet rays we have recently had occasion to feed cholesterol to rabbits for periods up to thirteen months and have observed xanthoma in the absence of trauma. In fact, xanthoma was produced whenever cholesterol was consumed for a long period, irrespective of other complicating factors.

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1 Verse, M. *Virchows Arch f path Anat* **250** 252, 1924. Berberich, J. *Klin Wchnschr* **4** 2335, 1925. Kusnetzowsky, N. *Virchows Arch f path Anat* **263** 205, 1927.

2 Anitschkow, N. *Arch f Dermat u Syph* **120** 627, 1914.

3 Schaaf, F. *Arch f Dermat u Syph* **175** 279, 1937.

## EXPERIMENTAL PROCEDURE

Fourteen young adult female albino rabbits weighing from 1,680 to 2,500 Gm were used. Eight of them were given a diet of Purina rabbit chow<sup>3a</sup> to which 0.23 per cent cholesterol and 3 per cent hydrogenated cotton seed oil (Crisco) had been added. Six controls were given only the Purina chow. The cholesterol was dissolved in hot Crisco, and this was poured over and thoroughly mixed with the pellets. The diet was consumed readily by the rabbits. Dried alfalfa was given twice weekly and water administered ad libitum. The animals were kept on shavings. When an animal died or was killed, sections were made of the liver, kidney, aorta and any pathologic tissue. Chemical analyses were made of the blood, liver, aorta and pathologic growths for cholesterol and fat. Six of the rabbits were irradiated sixty minutes daily with ultraviolet rays. After seven months Shope papilloma virus was injected into the ear vein or tattooed into the skin of the ear of each of 9 rabbits.

## RESULTS

Except 2 of the controls, which died during the second and third months, all the animals gained in weight during the first months of the experiment and remained in good health. The average daily consumption of food per rabbit was 83 Gm, equivalent to 190 mg of cholesterol. This is enough to cause cholesteremia and atherosclerosis but not enough to cause fatty liver<sup>4</sup>. After one month the cholesterol content of the blood of animals receiving cholesterol ranged from 3.58 to 12 mg per cubic centimeter of whole blood, with an average of 6.17 mg per cubic centimeter (table 1). In the control animals the average was 1.42 mg per cubic centimeter. Analyses made after ten and twelve months indicated that the hypercholesteremia was maintained in all animals consuming cholesterol regardless of treatment with virus or ultraviolet rays.

There was no detectable difference in weight, food consumption or general well-being between the irradiated and the nonirradiated animals. The ears of the irradiated animals, however, were slightly red. Those given virus responded as indicated in table 1. The virus warts were

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3a The formula for Purina rabbit chow as furnished by the Ralston Purina Company, St. Louis, is as follows: wheat germ, soy bean oil meal, corn germ meal, alfalfa leaf meal, wheat middlings, ground oats, corn meal, blackstrap molasses, calcium carbonate and iodized salt. All these are mixed thoroughly and then pressed into pellets. The chemical analysis is: protein, 16.45 per cent, fat, 4.06 per cent, fiber, 7.93 per cent, ash, 3.75 per cent, nitrogen-free extract, 52.60 per cent.

4 Baumann, C. A., and Rusch, H. P. *Proc. Soc. Exper. Biol. & Med.* **38**: 647, 1938.

usually confined to the ears, although some were scattered about other parts of the body

Subcutaneous nodules developed in the feet of 7 of the 8 rabbits receiving cholesterol, regardless of other treatments. Most of the nodules were noted after ten months of cholesterol feeding, but in 1 animal nodules developed after six months. The nodules continued to increase in size until the death of the animals. They were either single or multiple, and some were confluent. The footpads on all the feet were

TABLE 1—*Xanthoma Development in Rabbits*

Rabbit	Cholesterol in Diet	Ultra violet Irradiation	Virus Treatment	Response to Virus	Cholesterol in Whole Blood, Mg per Cc	Xanthoma	Comment
31	+	+	None		6.85	Large tumors on front and hindleg noted at 6th month	Died in 11 mo
32	+	+	Intravenous	Warts on both ears	5.25	Numerous nodules on all feet	Died in 13 mo
33	+	+	Intravenous	Few warts	5.30	Footpads enlarged	Died in 10 mo
34	+	+	Tattooed	Large local papilloma	6.18	Numerous nodules on both feet	Died in 12 mo
35	+	+	Intradermal	Few warts	3.58		Killed in 9 mo
36	+	+	Intravenous	Few small warts	5.03	Nodules on all feet	Killed for analysis at 12 mo
38		+	Intravenous	Warts on ears	1.46	None	
39		+	Intravenous	Warts on ears	1.52	None	
41		+	None		1.16	None	
42		+	None		1.53	None	
43	+		Intravenous	Few warts	5.20	Numerous nodules on all feet	Killed for analysis at 12 mo
44	+		Tattooed	Large local papilloma	12.00	Numerous nodules on all feet	Killed for analysis at 13 mo

involved, becoming swollen, slightly tender and denuded. Occasional nodules were observed also at the knees and elbows. The size of the tumors ranged from that of a pea to 6 by 7 by 8 cm. The average size was about 1 by 2 by 3 cm. The largest tumor occurred on the left front foreleg of rabbit 31, near the elbow, and completely incapacitated the affected leg. The animal was extremely emaciated and died during the eleventh month.

The tumors were soft, rounded and white or pale yellow. In gross appearance they strongly resembled the nodules in patients with xanthoma (fig. 1). Most of them were tough and firm on being cut but



Fig 1—Xanthoma nodules of rabbits' feet The foot without growths is that of a normal rabbit

contained soft areas from which a white smooth substance the consistency of thick pus could be extruded with slight pressure. Others were less firm and contained small amounts of milky fluid. The nodules on the pads of the feet were firmer than those on the top and sides. Deposits of a yellowish white material were firmly incorporated in and around the tendon sheaths.

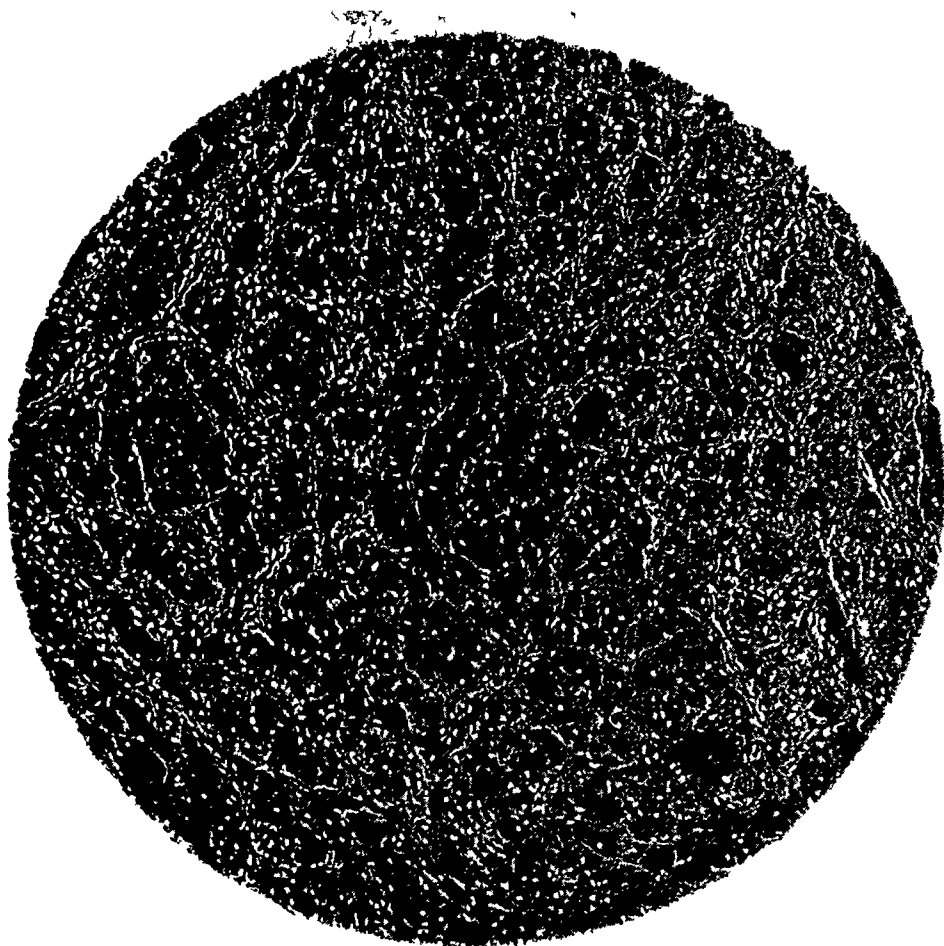


Fig 2—Photomicrograph of xanthoma nodule produced experimentally in a rabbit

Histologically, the tumors were composed of masses of large foamy cells (fig 2) loaded with lipoidal material. They appeared to be identical with the xanthoma nodules produced experimentally by Anitschkow and by Schaaf, and the foam cells, with the "xanthoma cells" or "cholesterol ester phagocytes" known in the human lesions.

Chemical analysis of 12 nodules taken from 3 rabbits indicated that fat constituted 16.6 per cent of the fresh weight or 46.4 per cent of the



dry weight of the tissue (table 2) The cholesterol content of the dried nodules ranged from 2.16 to 8.2 per cent, with an average of 4.57 per cent Approximately one half of the cholesterol was in the free form

Other changes accompanied the appearance of the nodules The animals began to lose weight, and the hair became scanty on the back, neck, abdomen and legs The underlying skin was wrinkled and had a yellowish cast Some of the animals also lost hair about the eyelids, the skin of which was baggy and wrinkled Numerous small white deposits were noted on the iris

The subcutaneous fat of animals killed after twelve or thirteen months differed from normal fat in that it was more granular and less greasy There was also marked atherosclerosis of the aorta One case

TABLE 2—Fat and Cholesterol Content of Xanthoma Tissue

Rabbit	Part Examined	Percentage of Fat Fresh Basis	Percentage of Fat Dry Basis	Percentage of Cholesterol, Dry Basis
32	Pad from front foot	19.8	41.8	3.94
	Pad from hind foot	21.6	48.4	5.45
	Nodule hind foot	35.4	64.0	8.2
	Axillary lymph node	10.7	30.6	5.5
43	Popliteal lymph node	6.5	36.0	4.1
	Pad from front foot	11.6	46.5	2.6
	Pad from hind foot	14.0	50.1	4.2
44	Pad from front foot	18.4	50.8	5.11
	Pad from hind foot	14.8	47.5	4.49
	Pad from hind foot	10.5	42.5	5.41
	Pad from hind foot	28.6	68.0	3.72
	Popliteal lymph node	7.0	30.5	2.16
Average		16.6	46.4	4.57

was noted in which this was so severe that all but one fourth of the normal opening was obliterated

COMMENT

While our experiments were complicated by the fact that the rabbits were irradiated with ultraviolet rays or inoculated with virus in addition to being fed cholesterol in their diet, it is nevertheless evident that the cholesterol was the essential factor in the formation of xanthoma nodules No effect due to ultraviolet rays could be demonstrated except a redness of the ears—which were not involved in the xanthomatous changes In rabbits 31 to 36 (table 1), which received ultraviolet rays, nodules developed as large and as numerous as those in rabbits 43 and 44, which were not irradiated In rabbits 38 to 42, which were irradiated but which received no cholesterol, xanthoma did not develop

Similar evidence excludes the virus as a cause of xanthoma Virus warts were confined largely to the ears, xanthoma nodules, to the feet

There was, however, some overlapping of effects, for virus warts were sometimes found on the legs while xanthomatous skin changes were sometimes general. Nevertheless there was no correlation between the response to the virus and the development of xanthoma. Xanthoma nodules developed in rabbits 34 and 44, which had large virus papillomas, exactly as in rabbits 33, 36 or 43, which had only a few transient virus warts (table 1). Rabbit 31, which received no virus, presented large xanthoma nodules. Rabbits 38 and 39 received virus but no cholesterol and did not show xanthoma nodules. Finally, the combination of the injection of virus and the ultraviolet irradiation failed to produce xanthoma.

The relationship between cholesteremia and human xanthomatosis has been pointed out frequently. Of 40 cases reported by 16 different authors, all but 10 were characterized by a definite increase in blood cholesterol.<sup>5</sup> In a case reported by Burns blood fat, as well as blood cholesterol, was increased.<sup>6</sup> Arning and Lippmann<sup>7</sup> demonstrated an increase in neutral fat, lecithin and cholesterol in the case described by them. However, many patients with diabetes mellitus show hypercholesteremia but do not have xanthoma nodules. This probably means that a high level of blood cholesterol must be maintained for a long period before growths will appear.

In the rabbit, certainly,\* a long period is needed for nodules to accompany cholesteremia. The level of cholesterol feeding employed by us results in hypercholesteremia in two weeks and definite atherosclerosis within a month, yet xanthoma nodules did not appear for ten months.

Several investigators have considered hepatic insufficiency as the cause of xanthoma because the lesions are sometimes associated with jaundice. Chauffard and Laroche<sup>8</sup> found that in 8 patients with icterus there was also an increase in blood cholesterol, but the two conditions appeared to be relatively independent. Thus the highest cholesterol content was found in a patient with merely a subicteric tint. Their tests for the cholesterol content of skin removed from patients with jaundice also were inconclusive. The attempt to correlate xanthomatosis with pancreatic disease has produced no real evidence.

Little is known regarding the genesis of the disease. Harrison and Whitfield<sup>5</sup> stated that it is a process of infiltration dependent primarily on hypercholesteremia and secondarily on the duration of the blood condition, local trauma and the state of the local vascular supply. Pollitzer and Wile<sup>9</sup> claimed that the "xanthoma cells" arise from the

5 Harrison, G. A., and Whitfield, A. *Brit J Dermat* **35** 81, 1923

6 Burns, F. S. *Arch Dermat & Syph* **2** 415, 1920

7 Arning, E., and Lippmann, A. *Ztschr f klin Med* **89** 107, 1920

8 Chauffard, A., and Laroche, G. *Semaine med* **30** 241, 1910

9 Pollitzer, S., and Wile, U. J. *J Cutan Dis* **30** 235, 1912

adventitial connective tissue cells of the smallest blood vessels of the papillary and subpapillary layers. As these cells become filled with cholesterol and increase in size and number they act as a stimulus to the connective tissue elements, fibroblasts and collagenous bundles, which surround the nests of xanthoma cells and ultimately predominate over the true xanthomatous elements. Basing his views on experiments with atherosclerosis in rabbits, Anitschkow<sup>10</sup> believes that the process attacks those parts which are composed of a dense framework of fibers, where the lymph circulates very slowly. The dense connective tissue, composed of various fiber systems with large surface area, acts as a barrier to the lymph flow with the result that the lipoids are arrested at these surfaces and undergo flocculent precipitation in some unknown manner. The lipoids are then phagocytosed by the lymphocytic and mononuclear wandering cells, which become the "xanthoma cells." Simultaneously fibrous proliferation occurs in this area. Our work as well as that of Anitschkow<sup>11</sup> demonstrates that xanthoma nodules occur principally in those parts which are composed of a dense framework of fibers. Because of the similarity here to the rabbit aorta, it appears that the production of "xanthoma cells" in other structures might occur in a similar manner.

#### SUMMARY

In rabbits receiving 0.23 per cent cholesterol in their diets for ten months xanthoma developed regardless of other treatments. Trauma is therefore not an essential prerequisite for the development of xanthoma.

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10 Anitschkow, N., in Cowdry, E. V. *Arteriosclerosis*, New York, The Macmillan Company, 1933, p. 298.

11 Anitschkow, N. *Charkowsky med j* **22** 30, 1916.

# SPONTANEOUS OPHTHALMIC MUTATION IN A LABORATORY MOUSE

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One of us (Dr Gowen) in the course of his studies observed a spontaneous ocular mutation in one of his mice. The animal was the result of a cross between two long inbred lines, lines which up to that time had been continued by brother and sister matings. The fact that the animals of each mating were examined carefully both for their own defects and for those of their progeny makes it probable that this appearance of the character was its first within the colony.

Extracted progeny from this animal showed characteristic changes in animals of either sex. The strain has been continued by mating defectives. All animals from these matings have been defective. Animals of either sex taken from this strain are slow breeders. Mice showing ocular defects have appeared in the progeny of no other matings, although there are twelve other closely associated strains in which the syndrome could appear.

The ocular change shows wide variations in its manifestations. Animals at 10 days of age may show microphthalmic eyes. At the other extreme animals may appear normal till 3 months of age, when a central opacity may appear in either eye. The eyes within the same animal may show variation, one eye may be microphthalmic, while the other is normal in size but opaque. The eyes of the same animal may differ in time of onset of the disease. Consequently one may say that the character is quite variable in its expression. The eyes of several mice were examined both grossly and by means of the corneal biomicroscope prior to the killing of the animals. After enucleation, hematoxylin and eosin-stained serial sections of the paraffin-embedded specimens were studied microscopically. Several photomicrographs were taken of a typical section of each eye of one animal for this report.

The skull and extremities presented no manifest abnormalities, and the gross examination revealed both eyes to be normally situated. The right eye appeared to be normal in size, and the lids showed no abnormality, however, the cornea presented a large central opacity, and

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the normal corneal reflex was lacking. The left eye was much smaller than normal, and the lids, particularly the margins, were occupied by approximating carrot-colored verrucous cornified masses. The cornea lacked luster and was very difficult to see through the small palpebral aperture. The left eye could not be examined with the corneal biomicroscope.

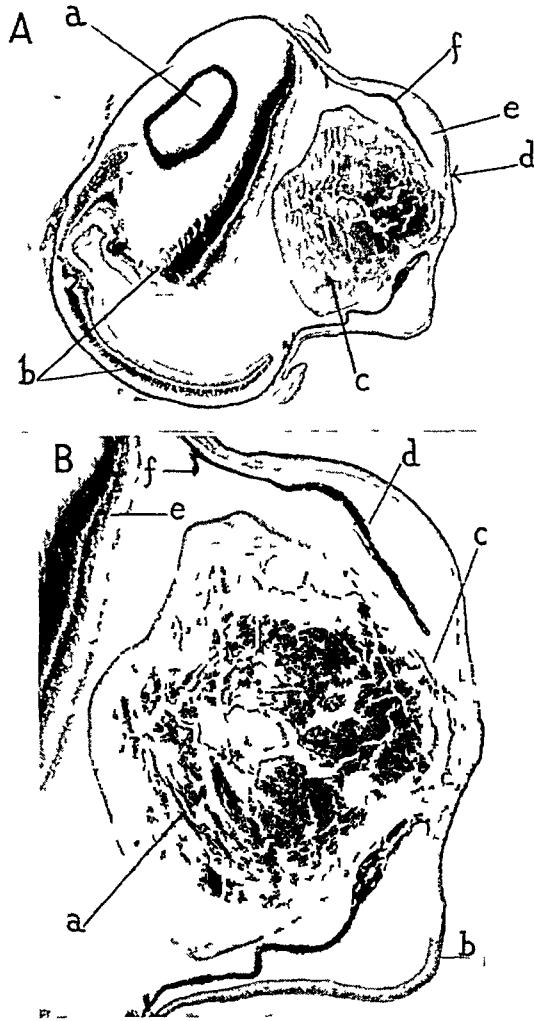


Fig 1—*A*, right eye,  $\times 16$  (a) artefactual invagination, (b) retina, (c) lens, (d) epithelium of the cornea, (e) anterior chamber and (f) iris. The relationship of the structures of the anterior segment is shown in this horizontal section. Posteriorly and above, the artefactual invagination of the sclera and choroid have resulted in a pushing downward and forward of the retina.

*B*, right eye,  $\times 32$  (a) lens, (b) epithelium of the cornea, (c) anterior capsule of the lens, (d) iris, (e) retina and (f) ciliary body. The rudimentary ciliary body, the heavily pigmented iris and the shallow anterior chamber are seen together with the intimate relationship of the lens and cornea.

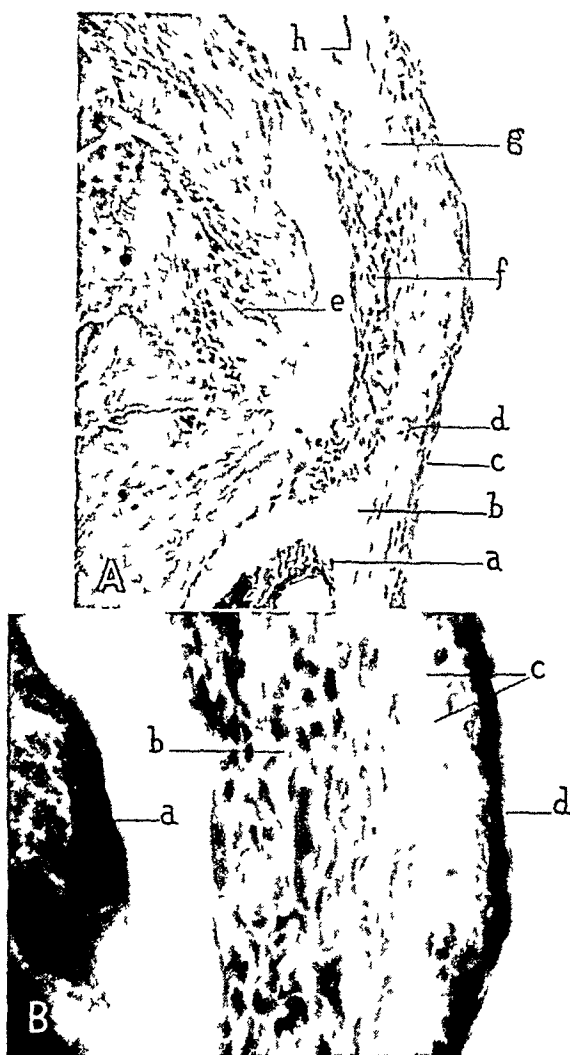


Fig 2—*A*, right eye,  $\times 123$  (a) iris, (b) anterior capsule of the lens, (c) epithelium of the cornea, (d) island of iris stroma and pigment cells, (e) lens, (f) anterior pyramidal cataract (proliferated subcapsular epithelial cells), (g) anterior capsule of the lens and (h) endothelium of the cornea. Small groups of pigmented iris cells are seen between the approximated posterior surface of the cornea and anterior surface of the lens. The anterior pyramidal cataract is seen as a bulging forward of the lens and consists of proliferated subcapsular epithelium. Below, the anterior surface of the iris meets the substantia propria and tends to continue forward and laterally (i.e., peripherally) along the substance itself.

*B*, right eye,  $\times 290$  (a) lens, (b) anterior pyramidal cataract (proliferated subcapsular epithelial cells), (c) anterior capsule of the lens and (d) epithelium of cornea. The homogeneous substance of the anterior capsule of the lens can be traced through the section as it lies in apposition to the thinned corneal substance. Behind the capsule lies the proliferated subcapsular epithelium. The corneal epithelium anteriorly is somewhat uneven in thickness. There is no evidence of infection.

The right cornea exhibited a large central opacification, which appeared to be connected intimately with the anterior surface of the lens. The periphery of the opaque region shaded off imperceptibly to clear, normal peripheral corneal tissue, behind which the anterior surface of a deeply pigmented structure, the iris, could be seen. Peripherally there was a very shallow anterior chamber, which was filled with clear

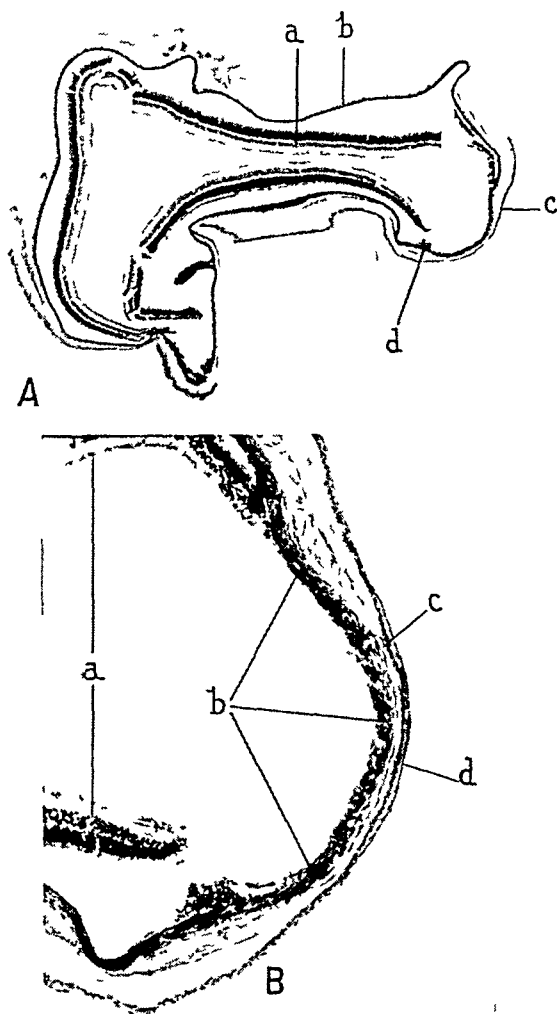


Fig 3—*A*, left eye,  $\times 17$  (*a*) retina, (*b*) sclera and choroid, (*c*) anterior surface of the cornea, the apex of which here is directed downward and somewhat to the right and (*d*) iris. Distortion has resulted from uneven fixation and paraffin impregnation. The anterior segment of the eye is seen to the right and is directed downward. The close relationship of the anterior structures is apparent.

*B*, left eye,  $\times 77$  (*a*) retina, (*b*) iris and pupillary membrane, (*c*) substantia propria of cornea and (*d*) epithelium of cornea. The eye is aphakic, there is no anterior chamber, the pupillary membrane is intact, the iris remains an embryonal structure and is adherent anteriorly to the cornea posteriorly. The ciliary body has not developed. There is no evidence of infection.

aqueous fluid. The leaf of pigmented tissue bowed forward to meet the posterior surface of the cornea at the junction of the latter with the anterior surface of the lens. The lens itself was rather opaque, and its deeper structures could not be observed in detail.

Microscopic examination of the serially cut sections revealed no evidence of previous intraocular infection. Typically, the posterior segment of the right eye exhibited no abnormality (figs 1 and 2). Anteriorly, there was an anterior pyramidal cataract with compact and opaque anterior fibers but without actual reduplication of the capsule itself. There was rather marked proliferation of the subcapsular epithelial cells. The capsule itself was thickened and compressed against the posterior surface of a defective cornea. The lamina elastica posterior and most of the substantia propria in the immediate region of the contiguous compression were lacking. The lamina elastica anterior was thinned and at some points had ruptured, and the cornea exhibited rather marked epithelial proliferation. Occasional islands of iris stroma and iris pigment cells were seen between the anterior capsule of the lens and the disorganized corneal structure. The ciliary body was poorly developed, and the partially distorted and heavily pigmented iris appeared to persist centrally to remain united in front of the lens. This was thought to be a persistent pupillary membrane. Intact corneal endothelium could be traced down posteriorly to be lost within the encroaching iris at the point where the normal pupil probably should have existed. The pigmented iris cells did not spread appreciably on the posterior surface of the cornea as the former came in contact with the latter structure.

Microscopic examination of the left eye revealed apparently congenital microphthalmos, aphakia, opaque cornea and complete persistent pupillary membrane (fig 3).

#### COMMENT

The defect in the right eye appears to have several components (1) a defective cornea, (2) a persistent pupillary membrane, (3) a poorly developed anterior chamber and ciliary body and (4) an anterior pyramidal cataract. There is no microscopic evidence of prenatal or postnatal intraocular infection. Similar defects, careful search of the available literature shows, have not been reported in laboratory mice, however, it is possible that certain gross defects in albino mice which Pearson<sup>1</sup> described briefly might be an exception to this statement. Bagg and Little's<sup>2</sup> mouse variants, irregularly inheritable types, pos-

1 Pearson, E. S. *Nature*, London **114** 433, 1924.

2 Bagg, H. J., and Little, C. C. *Am J Anat* **33** 119, 1924.



sibly induced by roentgen ray treatment of the mouse germ plasm, are other possible examples of defects showing similarity to ours. Rarely, profound malformations of the eyes are seen in instances of congenital cataract in man and, since similar anomalies have been bred pure in strains of rabbits, the defects unquestionably are inheritable (Friedenwald<sup>3</sup>). Mann<sup>4</sup> discussed the causes of congenital polar cataracts and of corneal developmental opacities in man and recalled the work of Collins<sup>5</sup>. The lack of information concerning the origin of congenital ocular defects in mice such as those reported here necessitates a search for an explanation by analogy among the known embryonic causes of similar defects in other vertebrates, particularly in man.

Supposititiously it appears that the defect in the right eye in this instance arose initially from a failure of the pupillary membrane to atrophy at the normal time during intrauterine development, i e., the abnormality was an involvement, in its first phase, of the postendothelial (mesodermal) tissue, which is a structure that is very marked in rodents. This would account for the subsequent poor development of the anterior chamber and of the ciliary body with concomitant continuation of the approximation of the cornea and the growing lens through pressure. Thus, each of the two latter structures would have caused in one another by means of their enforced contiguity the development of cataractous changes and loss of normal corneal structural development, with subsequent occurrence of opacities in the lens and in the cornea, respectively.

The defects in the left eye—microphthalmos and aphakia—permit only speculation concerning their origin. There was seen no anterior chamber, and the cornea showed little or no development of the substantia propria and of the lamina elastica posterior, in addition, there was intimate union of the corneal tissue, over its entire posterior surface, with the iris stroma. The ciliary body, as such, was only rudimentary. It is probable that not only was there a failure of normal development of the mesodermal tissue in this eye but there was also a failure of the corneal ectoderm to react normally during early intrauterine development to produce a lens plate.

It is difficult to offer an unequivocal explanation for the appearance of spontaneous defects such as these on the basis of only a few microscopic observations in one generation, although grossly the defect regularly appears in all mice of successive generations within this strain. Further evidence will be presented with the progress of different phases of the investigation.

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3 Friedenwald, J. S. *The Pathology of the Eye*, New York, The Macmillan Company, 1929.

4 Mann, I. *Developmental Abnormalities of the Eye*, London, Cambridge University Press, 1937.

5 Collins, E. T. *Tr. Ophth. Soc. U. Kingdom* **18** 124, 1898.

# MEMBRANE FORMATION AT LIPOID-AQUEOUS INTERFACES IN TISSUES

## II A CORRELATION OF THE MORPHOLOGIC AND CHEMICAL ASPECTS

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Previous studies have shown that several liquid soluble unsaturated organic compounds which are relatively immiscible with aqueous tissue phases undergo gradual conversion into insoluble amorphous materials during a period in the subcutaneous tissues of guinea pigs<sup>1</sup> Under appropriate conditions this conversion results in the formation of continuous membranes at the interface between the residual liquid compounds and the limiting tissues These membranes resemble some naturally formed structures<sup>2</sup> They offer an opportunity for investigating the gradual formation of complex interfacial films which can be visualized microscopically as they develop under biologic circumstances

The study of these membranes has been conducted with four principal objectives in mind The first objective was the distinction of a most elementary type of chemical structure, which was susceptible to the biologic mechanisms responsible for the transformation As a solution of this part of the problem, data have been obtained which indicate that the simplest required chemical structures were long normal carbon chain acids or acyl groups with at least two ethylenic linkages in the chain<sup>1</sup> A larger number of ethylenic linkages distinctly favored the transformation These structural configurations alone were adequate for conversion but in the simplest case were not sufficient for the formation of long continuous interfacial membranes The second objective, therefore, was the defining of an elementary chemical structure which was adequate not only for support of the biologic transformation but for support of membrane formation In an approach to this problem, data have been obtained which indicate that some chemical alterations which accompany mild oxygenation of long normal chain highly unsaturated acids or acyl groups supply the added structural configuration which leads to the formation of continuous interfacial membranes<sup>2</sup> Further efforts to

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1 Hass, G M Arch Path **26** 1196, 1938

2 Hass, G M Arch Path **27** 15, 1939

define the general nature of these alterations have been made, and the results will be reported on subsequent pages

The preceding data have indicated general lines of procedure for approaching the third objective, namely, a determination of the fundamental structure of these interfacial membranes, as they are constructed in the biologic environment under investigation. Data pertaining to this aspect of the problem will be presented in this report.

The final step in this investigation follows in natural sequence and will encompass all attempts to formulate such chemical and morphologic rules of behavior of compounds in this semibiologic system as might be applied to natural systems under more nearly physiologic circumstances.

Among several possible approaches to this aspect of the problem, the following have been made, and the experimental results will be recorded here. First, extracts of several mammalian tissues have been prepared for purposes of demonstrating whether materials amenable to the transformation are present in normal tissues. Second, two representative classes of natural esters, cholesterol esters and lecithin, which often have highly unsaturated acyl groups, were added to the list of compounds under investigation. Third, experiments were devised to determine whether the transformation is possible in bodily sites other than the one previously investigated. Fourth, attention was directed to the possible participation of protoplasm in the structure of the membranes.

#### METHODS

Experiments designed to compare the transformation of acid-free cod liver oil in the subcutaneous tissues of guinea pigs with the behavior of the oil in other tissues were done as follows. Acid-free cod liver oil (0.2 cc) was injected directly into the spleen, liver, kidney, pancreas and testis. Intravascular injections were made by way of the ventricles of the heart and the spleen. The intraventricular injections were always followed by death of the animal within a few hours and consequently were unsatisfactory. Intrasplenic injections were consistently satisfactory and yielded an opportunity to study the transformations of the oil in the spleen, liver and lungs, the oil having been transported from the spleen to the liver and lungs by the vascular route. The experiments were terminated after intervals of two or three weeks. The tissues were fixed in solution of formaldehyde U S P (10 per cent), and representative blocks were embedded in paraffin. Hematoxylin and eosin, Weigert's stain for elastic tissue and Ziehl-Neelsen carbolfuchsin stain were used routinely on paraffin sections.

Preparations of materials from mammalian tissues for the purpose of demonstrating the presence of compounds amenable to the transformations in question were made as follows. Fresh guinea pig brains and livers and human brain tissue were used. These tissues were macerated in separate containers and then extracted with alcohol. After several days the residue was separated by filtration and extracted with peroxide-free ether for forty-eight hours. The ethereal extract in each instance was concentrated until the solution was highly viscous. The subcutaneous injections and the preparation of tissue for microscopic study were made in the usual manner.

Blood was obtained from 4 different persons. Immediately after 25 to 50 cc of blood was drawn by venous puncture, it was placed in acetone. After several hours the acetone was removed by filtration, and the residue was extracted for at least twenty-four hours with ether. The acetone and ether extracts were combined and evacuated to dryness. The dried material was taken up into a small amount of purified petroleum benzine and injected in solution. The injections were made subcutaneously, and the tissues were prepared for study by methods previously described.

As cholesterol esters of unsaturated long chain organic acids which can be conveniently purified are solid at 37.5 C, liquid cholesterol esters were prepared as follows. Fifteen grams of highly unsaturated acids of cod liver oil obtained by methods previously described was mixed with 10 Gm of cholesterol (melting point 145 to 146 C). The solution was agitated gently in the presence of a slow stream of dry hydrochloric acid at 35 C for ten minutes. Free hydrochloric acid was evacuated, and the mixture was then heated in an atmosphere of carbon dioxide for one hour. The products were taken up in ether. The ethereal solution was washed free of acids with dilute aqueous potassium hydroxide. The ether was distilled off. The products were fractionally crystallized from alcohol over a period of several days, a small amount of water being added to the alcohol after the removal of each crop of crystals. Finally, a residue of about 1.5 Gm of liquid esters was obtained. This mixed product formed liquid crystals. It began to solidify at about 30 C and was almost entirely solid at 15 C. Cholesterol and liquid fatty acids in amounts in accord with approximate calculations were obtained by hydrolysis of a small amount of the material. This liquid product was injected subcutaneously in doses of 0.2 cc. After intervals of two or three weeks, the tissues were prepared as usual for study.

Lecithin was purified and administered in the following manner. Ten grams of "egg lecithin" (Merck) was dissolved in alcohol. An excess of cadmium chloride was added to the solution. The precipitate was thoroughly refluxed in ether. The ether-insoluble material was dissolved in a mixture of boiling ethyl acetate and alcohol. On cooling, the cadmium chloride-lecithin addition compounds precipitated. The precipitate was removed by centrifuging and subsequently dissolved in chloroform. The lecithin was set free from the cadmium chloride by addition of ammonia in alcohol to the chloroform solution. The precipitate was removed by centrifuging. After evaporation of the chloroform, the lecithin was dissolved in ether and precipitated quantitatively with acetone. The lecithin so obtained was almost pure white, soft, plastic and highly hygroscopic. After the lecithin had been dried in high vacuum, saturated solutions of lecithin in high-boiling paraffin oil, olive oil and butyl oleate were prepared. Two-tenths cubic centimeter of each saturated solution was injected subcutaneously into guinea pigs. After intervals of two or three weeks the tissues were fixed and stained in the routine manner.

The study of the relation of tissue proteins to the lipoidal membranes was conducted by microscopic examination of a considerable amount of material available. In addition to the stains mentioned previously, Mallory's aniline blue-orange G stain for connective tissue, osmic acid stains of fresh tissues and the Swank-Davenport modification of the Marchi stain for degenerating myelin were used.

The influence of several common oxidizing and reducing agents was studied in the following manner. The reducing agents employed *in vivo* were hydroquinone

and pyrogallol. Each of these compounds was added (2 to 5 mg per gram) to separate samples of acid-free cod liver oil and to oxygenated methyl esters of unsaturated acids derived from cod liver oil. The oil and mixed esters, each containing a reducing agent in solution, were injected subcutaneously. Appropriate control experiments were done. The animals were killed at the end of two or three weeks. The tissues were treated in the routine manner.

The study of the action of oxidative catalysts *in vivo* was made as follows. Cod liver oil (acid-free), methyl esters of unsaturated acids derived from cod liver oil and oxygenated methyl esters of unsaturated cod liver oil acids were employed. Various finely pulverized ferric salts (ferric chloride, ferric stearate and ferric oleate) and ferrous chloride were added to samples of each material, and after centrifuging, the oil and esters containing iron salts in solution and colloidal suspension were injected subcutaneously. The tissues at the end of ten days, two weeks or three weeks were treated in the routine manner. An additional stain, the prussian blue stain for iron, on sections cut from paraffin was used in this series of experiments.

In previous studies several comments have been made with regard to ferric chloride-oxygen treatment of the liquid unsaturated esters *in vitro* and concerning permanganate oxidation of fixed and mounted preparations. An extension of the study of the action of oxidizing agents *in vitro* was conducted by direct fixation of fresh tissues in 1 per cent aqueous osmium tetroxide (perosmic acid) and by employing the Swank-Davenport modification of the Marchi method on tissues fixed in solution of formaldehyde. Acid-free cod liver oil, oxygenated methyl esters of unsaturated acids of cod liver oil and linseed oil plus 20 per cent unsaturated acids of linseed oil were injected into guinea pigs subcutaneously. After intervals of two or three weeks, selected blocks of tissue at the sites of injection were prepared for study by direct fixation in 1 per cent perosmic acid for twenty-four hours, followed by rapid dehydration and paraffin embedding. Adjacent blocks of tissue were fixed in solution of formaldehyde and prepared according to the Swank-Davenport method for staining degenerating myelin.<sup>3</sup>

As unsaturated methyl esters of cod liver oil acids do not form continuous membranes in the tissues unless they have been subjected previously to oxygenation, a preliminary investigation of the oxygenated materials was conducted, with the idea of demonstrating the creation of polar groups at points of unsaturation as the result of partial oxidation. For this purpose, methyl esters of unsaturated acids of cod liver oil (50 Gm) were oxygenated by bubbling air through them for seven days. The products assumed a yellowish tinge and became increasingly viscous, some materials either settling to the bottom or adhering to the glass wall of the oxygenating apparatus. All dissolved easily in ether. The ethereal solution was washed with aqueous potassium hydroxide, and after the ether was distilled off, the quantity was divided into two equal parts. One was treated in the usual way with phenylhydrazine and acetic acid, chloroform being used as the solvent. The other was treated with benzoyl chloride, pyridine being used as the required base and chloroform as the solvent. There were appropriate controls. In each instance, after concentration of the reacting mixtures and addition of benzene, a crystalline precipitate was obtained. The compounds which were regenerated by hydrolysis of the hydrazones and benzoyl derivatives were brownish liquids. They were unstable and became so altered during the manipulations that they were unfit for further dependable study.

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3 Swank, R. L., and Davenport, H. A. *Stain Technol* **10** 87, 1935

Recourse, therefore, was made to an application of methods for demonstrating carbonyl and hydroxyl groups of compounds in solution to this problem, in which I had to deal with insoluble materials in paraffin sections of tissues mounted on glass slides. Phenylhydrazine, phenylhydrazine hydrochloride and hydroxylamine hydrochloride were selected as reagents for the carbonyl group. Benzoyl chloride and acetic anhydride were chosen as reagents for possible condensation with hydroxyl groups. Chloroform or alcohol was used as a solvent for the reagents. After immersion of thin sections of tissue mounted on glass slides in the solvents, the specific reagents and adjunctive reagents were added. The methods were the same as those generally in use in work with materials in solution and will not be discussed fully here. The preparations were allowed to stand at room temperature in the dark for periods varying from three to twenty-four hours. The slides with tissues mounted on them were then washed in alcohol and water. The tissues were subsequently stained by Weigert's resorcinol-fuchsin method. The membranes were studied microscopically and the intensity of election of the resorcinol-fuchsin stain graded directly by color vision.

Two types of membranes were investigated by these methods, one arising by transformations of cod liver oil and the other by conversion of oxygenated methyl esters of unsaturated acids of cod liver oil. Eleven experiments have been completed on sections of tissue mounted in series. The results include data on the influence on the intensity of election of Weigert's resorcinol-fuchsin stain by membranes treated as follows: (1) by a "keto-fixing" reagent, (2) by a "hydroxyl-fixing" reagent, (3) by a "keto-fixing" reagent followed by a "hydroxyl-fixing" reagent and (4) by solvents and other adjunctive reagents necessary for making the determinations.

#### EXPERIMENTAL RESULTS

Cod liver oil (acid-free) after direct injection into the spleen, liver, pancreas, kidney and testis was partially transformed into the characteristic insoluble semisolid materials. If the cod liver oil was introduced intravascularly by way of the splenic sinuses, the same general types of alterations occurred and were noted in the splenic pulp, the liver and the lungs. In the several sites of localization there were minor differences in the rate and extent of transformation of the oil. The opportunities for the persistence of long continuous lipoid-aqueous interfaces in the lungs, liver, kidney and spleen were of course greatly restricted. Perfectly formed membranes entirely similar to those described previously in the subcutaneous tissues were routinely found at extended lipoid-aqueous interfaces in the pancreas and testes. In no instance were the altered lipoids demonstrable in epithelial or mesenchymal cells other than those which are generally believed to have phagocytic capacities. In general, extracellular transformed products were easily demonstrable, and all studies indicated that intracellular conversion also occurred in the same way as in the subcutaneous tissues.

The experiments with extracts of human brain and whole blood as well as those with extracts of guinea pig brain and liver showed that in the ether-soluble and acetone-ether-soluble fractions as prepared there were materials which underwent alterations analogous to those under

discussion The altered products were not abundant and were always present in the form of small globules and granules No long continuous lipid-aqueous interfaces were seen in the tissues, so that no conclusions could be drawn with respect to the presence of compounds suitable for transformation into membranes at such boundaries The principal part of the insoluble materials which elected Weigert and Ziehl-Neelsen stains was in the cytoplasm of macrophages and multinucleated giant cells

At the sites of injection of liquid cholesterol esters of unsaturated acids of cod liver oil there was a severe inflammatory reaction with extensive necrosis of tissues Clefs in which cholesterol crystals were embedded were common These clefs were often marginally limited by multinucleated giant cells These details indicated that partial hydrolysis of the esters had occurred during their residence in the tissues There was a fairly large amount of insoluble material derived either by transformations of the esters or their products of hydrolysis This material was acid fast and took Weigert's resorcinol-fuchsin stain It was principally in the form of small globules and granules, although occasionally long continuous delicate interfacial membranes were encountered These resembled those which have been described previously around residual deposits of oxygenated methyl esters of unsaturated acids of cod liver oil

No insoluble material which elected Weigert's stain or exhibited acid-fast properties by the Ziehl-Neelsen method was found at the sites of injection of purified egg lecithin

The protoplasmic components which were most frequently present on the aqueous aspect of the lipid-aqueous interface were collagen, fibrin and cytoplasm The relation of these elements to the membranes may be described as follows

Homogeneous or fibrillar collagen was occasionally the only distinguishable morphologic element on the aqueous aspect of the interface (fig 1 *A*) This was not only preformed collagen but also that which had been deposited during the period of membrane formation A line of demarcation between the altered lipoids and the limiting collagen could not always be distinguished In other words, there was a narrow interfacial region where fusion of liquid or fibrillar collagen and altered lipoids seemed to occur In such areas the membranes were sharply and deeply stained on their lipoidal aspect This sharp staining, illustrated best by Verhoeff's stain for elastic tissue, gradually became less intense as the interface was approached and merged into the counter-stain characteristic for the limiting collagen These relations were also clearly shown with Mallory's aniline blue stain for connective tissue Normal collagen was stained blue as usual The principal part of the membrane was stained yellow At points of fusion of the yellow altered lipoidal components of the membranes with the underlying blue collagen,



Fig 1—*A*, section showing transformation of oxygenated methyl esters of highly unsaturated acids of cod liver oil plus a ferric chloride catalyst in subcutaneous tissues for two weeks, formaldehyde fixation, Weigert stain for elastic tissue. The clear space represents the site of the soluble residual components of the ester globule. The wavy black structure (about 8 to 10 microns thick) is the membrane which has formed at the interface between the ester and the adjacent aqueous protoplasm. Note the limiting fibrillar collagen at the base of the membrane. The delicate black fibrils which extend from the region of the interface in the plane of the collagenous fibrils may be preformed elastic fibrils, but there are no readily applicable methods which may be depended on to distinguish them as wholly independent of the interfacial membrane. *B*, section showing alterations of oxygenated methyl esters of highly unsaturated acids of cod liver oil in tissues for four weeks, formaldehyde fixation, Weigert stain for elastic tissue. The clear space is the site of residual esters which have been dissolved by the treatment of the tissues with alcohol and chloroform. This photomicrograph illustrates the highly convoluted continuous membrane which has formed at the ester-protoplasmic boundary. The underlying collagen, which by the method used stains gray rather than black, which is characteristic for the membrane, is closely applied to the base of the membrane and is intimately fused with it, extending into the numerous folds so as frequently to be attenuated to the point of invisibility.





Fig 2—*A*, section showing the effect of acid-free cod liver oil in the subcutaneous tissues for two weeks, formaldehyde fixation, Verhoeff's stain for elastic tissue. The clear space above the curved margin represents the site of the soluble components of the oil globule. The marginal grayish black zone (about 5 or 6 microns in breadth) is the interfacial membrane. This is sharply limited basally by a continuous layer of cytoplasm. Note the condensation at the line of contact between the cytoplasm and the interfacial membrane. *B*, reaction showing effect of acid-free cod liver oil in the tissues for two weeks, formaldehyde fixation, Verhoeff's stain for elastic tissue. The clear space is the site of residual soluble oil. The highly complex margin shown in this photograph was initially the curvilinear margin of a spherical globule of oil. This illustration shows, as well as can be shown within the limits of photographic reproduction, the arborescence of limiting protoplasm at the interface. Each delicate protoplasmic projection is covered by a thin layer of the lightly stained interfacial membrane.

a narrow zone of greenish coloration was often noted. This region, as a rule, remained optically homogeneous, and only rarely were collagenous fibrils demonstrated as a basal component of the membranes. The fibrils were principally oriented as limiting structures on the aqueous aspect of the interface. As such they were often firmly bound to the membranes and remained adherent to them where the membranes had been mechanically dislocated from their site of formation. Therefore, in some areas the following layers were distinguished: first, the peripheral zone composed of altered lipoids, second, a narrow zone of altered lipoids fused with collagen, third, a layer of fibrillar or liquid collagen, and fourth, a limiting margin of mesenchymal cells.

This arrangement was also detectable in perosmic acid and Swank-Davenport preparations. In these sections thin films of the artificially introduced compounds were occasionally present between delicate collagenous fibrils at the boundary. These were visualized in two dimensions as delicate layers of lipoid alternating with protein (fig. 3*A*).

The participation of collagen in the structure of membranes at the interface was always greatly limited. It seemed that surface forces and simple restricted diffusion would account for most of the microscopic picture, although there were no reasons for denying the probability of formation of lipoprotein compounds by chemical interaction.

The preceding account of the participation of collagen in the structure of the membranes was in general applicable when fibrin was concentrated on the aqueous aspect of the interface.

The interpretation of the histologic picture when cell margins formed a limiting boundary at the interface was difficult. The margins of cells, especially elongated fibroblasts and multinucleated giant cells, were usually sharp and well defined (fig. 2*A*). In other instances the cytoplasm was apparently so fused with the altered lipoidal compounds that no cell membrane was distinguishable (fig. 3*B*). This apparent peripheral coalescence of protoplasm with the altered lipoids at the interface was demonstrable with all types of stains used. Not infrequently a line of cleavage was present, so that the marginal protoplasm was detached from the body of the cell and remained bound to the lipoidal component of the membrane. All data favored the conclusion that the lines of cleavage were artificially produced by trauma incidental to the preparation of tissues for study. Nevertheless, there was no hesitancy in concluding that peripheral cell protoplasm was occasionally so modified by its contact and fusion with altered lipoids that it formed, from the histologic point of view, a basal component of the membrane.

The binding of collagen, fibrin and cell protoplasm to the altered lipoids of which the membranes were principally composed resulted in several interesting architectural arrangements of lipoid and protein. Over portions of the membrane boundary the proteins formed a



Fig 3—*A*, section showing the effect of methyl esters of unsaturated acids of cod liver oil in the subcutaneous tissues for two weeks, direct fixation and staining with 1 per cent aqueous osmium tetroxide. This field shows clear areas from which residual esters have been dissolved during preparation of the tissues and black areas of residual esters which have become insoluble in the dehydrating agents and which have retained their capacity to reduce osmium tetroxide. Note the thin black films of lipoids which extend between delicate collagenous fibrils for variable distances from the three major ester-protoplasmic interfaces shown in the photograph. *B*, section showing the effect of oxygenated methyl esters of unsaturated acids of cod liver oil in subcutaneous tissues for three weeks, formaldehyde fixation, Swank-Davenport modification of the Marchi stain. The clear space at the upper margin represents the site of residual esters which remained soluble in the dehydrating agents despite the treatment with the perchlorate-perosmic acid solution. Adjacent to this clear area is a discontinuous black zone of esters which reduced perosmic acid and remained insoluble in the dehydrating agents. Beneath this interrupted layer there is a pale gray interfacial membrane, which has largely lost its capacity to reduce perosmic acid. This section of membrane is representative of certain segments which cannot be clearly demarcated from the cytoplasm of adjacent cells (in this instance a multinucleated giant cell).

tangential marginal component which was either homogeneous or fibrillar (fig 1 *A*) Over other portions of the boundary the protein formed a tangential component composed of fibrils alternating with thin films of lipid (fig 3 *A*) Over still other parts the membranes were often convoluted and wavelike on cross section (fig 1 *B*) The lengths and amplitude of the waves varied greatly Where wavelengths were short and amplitudes great, the proteins at the boundary so conformed that in the extreme case they were disposed in a direction transverse to the general plane of the membrane and tangential to the plane of the front of the waves At times the protein so included was attenuated to the point of invisibility (fig 2 *A*) Whether it disintegrated or became fused wholly in the membrane was a question not solved by the methods employed The arrangement which has been described occurred irrespective of the protein implicated

Hydroquinone or pyrogallol if dissolved in compounds prior to injection into the subcutaneous tissues did not alter either the rate or degree of transformation of the compounds *in vivo* Neither did either material interfere with the mechanisms responsible for the formation of membranes

The presence of ferric or ferrous compounds in the materials during their residence in the subcutaneous tissues resulted in pronounced acceleration of the transformation of the various unsaturated compounds *in vivo* This was independent of the nature of the iron-bearing compound used In general, the extent of alteration in the presence of iron salts at the end of ten days or two weeks compared favorably with the degree of conversion of control materials at the end of three or four weeks The morphologic end result of the transformation in each instance was essentially the same

The distribution of iron as demonstrated by the prussian blue stain on paraffin sections was variable The relation of iron to the intracellular transformed compounds and to the extracellular long continuous membranes was of interest In macrophages and multinucleated giant cells the pattern of distribution of iron demonstrated with prussian blue often coincided with that which had been observed in occasional zones of reaction previously studied, in which no iron had been added to the compounds employed In this instance, it was assumed that the iron was derived principally from disintegrating hemoglobin

The continuous membranes showed in many areas a type distribution of iron which was sufficiently frequent to warrant comment In the region of the junction of the lipoidal aspect of the membrane with the limiting cell margins a row of iron granules was seen A similar planar distribution of iron was occasionally demonstrated on the opposite margin of the membranes When both distributions of iron were present

in the same segment of membrane, the altered lipid appeared to be encased in a more or less continuously distributed sheath of iron. In addition to these distinct linear arrangements, a delicate diffuse blue staining of the membranes was occasionally noted. This was so prominent in several sections of membrane that it was detectable by low power microscopic examination. Iron otherwise was distributed at random, although in the cytoplasm of phagocytic cells it was often in association with ingested lipoidal granules.

The action on the altered lipoids of aqueous osmium tetroxide (1 per cent) or aqueous osmium tetroxide in solution with formaldehyde, potassium perchlorate and acetic acid (as required in Swank and Davenport's modification of the Marchi method) may be described as follows. Although there were minor differences according to the method used, these have not been carefully worked out. The general results may be considered as similar for my purposes at this time. The compounds which were used were of such nature initially as to effect a reduction of osmium tetroxide by either method. In the course of biologic conversion of the compounds they gradually lost their initial capacity for reducing perosmic acid. This was more satisfactorily demonstrated by the Swank-Davenport method. By either method a part of the residual compounds which lay adjacent to the membranes was converted so as to be resistant to the action of the solvents employed in preparation of the tissues. Thus these preparations showed, first, the internal materials which were stained black by the reduction compounds of perosmic acid and, second, the marginal materials which had either partially or completely lost this reducing power (fig 3 B). These sections of membranes, filaments, globules and granules lightly retained the fuchsin counterstain.

The methyl esters of unsaturated acids of cod liver oil after seven days' oxygenation *in vitro* contained liquid soluble compounds which formed crystalline derivatives with both phenylhydrazine and benzoyl chloride. The yield of crystalline derivatives was small in each instance. On hydrolysis of these compounds a viscous dark liquid which was unfit for further experimentation was obtained. Although these observations indicated that keto and hydroxyl groups had been created by oxygenation of the unsaturated compounds, the small yields and instability of the regenerated products were strong arguments against this approach to the problem of chemical groups operating in the biologic formation of membranes.

The results of chemical tests which were made on the lipoids in thin sections of tissue mounted on slides were as follows.

A treatment with phenylhydrazine, phenylhydrazine hydrochloride or hydroxylamine hydrochloride produced such alterations as to bring

about an inhibition of the election by the membranes of the resorcinol-fuchsin stain. In some instances the inhibition was practically complete. Unfortunately, phenylhydrazine and its hydrochloride gave lightly colored yellowish solutions under the experimental conditions. The membranes acquired a similar color during the treatment, and this interfered with the interpretation of the intensity of staining by Weigert's method. If this yellowish coloration was a consequence of direct dyeing, the altered lipoids were the only components in the tissues which were so stained. Hydroxylamine hydrochloride did not give colored solutions in these experiments, and the results from its use served as a fairly satisfactory check on the interpretation of results when sections were treated with phenylhydrazine or phenylhydrazine hydrochloride.

The results of attempts to interfere with resorcinol-fuchsin staining of altered lipoids by a previous treatment with benzoyl chloride or acetic anhydride were not so satisfactory. There was constant demonstrable inhibition of election of the stain by lipoids subjected to the action of these reagents, but it was invariably slight.

The treatment first with a "keto-fixing" reagent and second with a "hydroxyl-fixing" reagent usually but not always diminished the inhibition obtained by use of the "keto-fixing" reagent alone.

Many controls were tested simultaneously. The only accessory reagent or solvent that interfered with the resorcinol-fuchsin reaction was sulfuric acid. This was used at first in small amounts as a catalyst for acetylation but was abandoned in later experiments. This intensified the resorcinol-fuchsin staining to such an extent that even some collagenous fibrils tenaciously retained the stain. This occurred when sulfuric acid was added to control solutions as well as when it was used as a catalyst in the experiments with acetic anhydride.

#### COMMENT

The first three sets of data with which I am concerned here require little comment, for they are in accord with prediction based on previous studies.

First, all previous studies had been conducted on materials introduced into the subcutaneous tissues. Data have been presented in this report which show that the transformation in question occurs in various other sites in the body. There were many minor variations in the rate and end result of the transformation in the different localities. These will not be discussed, for they have no immediate bearing on the problem. Suffice it to say that insoluble products of the conversion were always either in the intercellular phase or within the cytoplasm of phagocytic cells and never within the cytoplasm of epithelial cells.

The second set of data proves that mammalian tissues contain ether-soluble or acetone-ether-soluble materials which undergo the transforma-

tion These materials were found in each tissue under investigation, namely, human brain human blood, guinea pig brain and guinea pig liver The products of transformation were either granular or globular and were not abundant in any instance They elected the Ziehl-Neelsen and the resorcinol-fuchsin stain, as demonstrated previously with the products of transformation of unsaturated compounds from non-mammalian sources Further manipulation of tissue extracts, with eventual isolation of the more highly unsaturated components, would certainly improve the results of this study, and the findings might be of considerable interest

The third set of data aids in a further naturalization of the biologic transformation under discussion Previous investigations have principally been restricted to esters of unsaturated long chain organic acids with monohydric alcohols or glycerol The data presented here again demonstrate the importance of the acyl radical, for cholesterol esters yielded products of transformation similar to those previously obtained by the use of oxygenated methyl esters of the same group of unsaturated acids There was evidence of partial hydrolysis of cholesterol esters during their residence in the intercellular region Therefore, there was no absolute proof that cholesterol esters as such were transformed, although if past experience with other esters can be called on for support of an opinion, the direct conversion of cholesterol esters independent of hydrolysis probably occurred There was little doubt that some mild oxygenation of the unsaturated acyl groups accompanied preparation of the esters, it is not entirely proper to state that continuous membranes formed at cholesterol ester-tissue interfaces in the absence of preliminary oxygenation such as was necessary for membrane formation by methyl esters of the same group of unsaturated acids This may serve as an object for further study because of the general similarity of transformations of highly unsaturated liquid cholesterol and methyl esters

The purified egg lecithin introduced in a variety of solvents yielded no insoluble material which was stained positively by the Weigert or the Ziehl-Neelsen method The result is contrary to prediction There is no explanation for the negative result Perhaps more highly unsaturated natural lecithins, such as those obtainable from liver or brain, might be more satisfactory products for study

Microscopic studies showed frequent participation of protoplasm in the basal structure of the membranes at the lipid-aqueous interface The proteins which were demonstrated as definitely contributing to the basal structure of some areas of continuous membranes were collagen and fibrin The implication of proteins seemed to depend principally on restricted interpermeation of lipoids and proteins at the interface as well as on surface adhesion The interpermeation was illustrated by the presence of delicate films of lipid which extended from the inter-

facial region to pass between limiting strands of collagen and fibrin. The adhesion of well defined proteins to the altered lipoids of the membranes was everywhere in evidence and usually was independent of demonstrable interpermeation. This appeared to be of the nature of a strong surface attraction, although the chemical binding of altered lipoids to limiting proteins at the interface must be considered as a likely possibility.

The implication of the marginal protoplasm of mesenchymal cells at the interface in the basal structure of membranes presents some interesting problems. The conclusion that cytoplasm may be fused with the altered lipoids arose from two morphologic facts. First, in rare instances no sharp line of separation between marginal cytoplasm and adherent lipoidal membranes could be distinguished. Second, lines of intracytoplasmic cleavage were occasionally noted where membranes in the course of mechanical separation from their site of formation had been detached. In these areas marginal cytoplasm remained intimately fused with the detached membranes. Thus, in several instances an effective marginal cytoplasmic block was produced by some interaction between cytoplasm and adjacent altered lipoids. This is emphasized not only because it is illustrative of the participation of protoplasm in the basal structure of portions of membranes but because it may be a useful experimental device for studying controversial questions related to histogenesis of intercellular materials at cell margins.

Another aspect of the participation of tissue proteins in the structure of membranes was a consequence of the tendency of the membranes to assume a folded or wavy contour. Under these circumstances the tissue element involved at the interface accompanied the convolutions. As the amplitude of the folds was increased and as the distance between their crests was decreased by lateral condensation, the protoplasmic component—fibrin, collagen or cytoplasm—became attenuated and normally oriented to the general plane of membrane boundary. With continued condensation the delicate protoplasmic invaginations became attenuated to the point of invisibility. The fate of the proteins so included could not be determined. As a rule, the prolongations retained a basal connection which in some instances was obliterated either by compression or by detachment of membranes from their site of formation. This mechanism gives further insight into the physical possibilities of interfacial behavior under biologic circumstances. With large numbers of thin films of this general type, the surface forces would be great indeed and conducive to high elasticity and tensile strength. From this model many variations depending on the distribution of the lipid with respect to the protein are of course realizable, and these data give only the simplified relations which exist between a continuous lipoidal film and a limiting, more or less continuous protein film.



It has been shown previously that methyl esters of unsaturated acids of cod liver oil as obtained by high vacuum distillation were not transformed into long continuous membranes at lipid-aqueous interfaces in the tissues. If the esters were oxygenated at room temperature for several days, they acquired the property by which they were amenable to transformation into insoluble continuous membranes at the interface between residual liquid-soluble compounds and the aqueous limiting tissue phase. It has been accepted for some time that such treatment of highly unsaturated long chain acyl groups results in the addition of oxygen at the double bonds with the formation of peroxides.<sup>4</sup> These peroxides are unstable and are supposed to rearrange into hydroxy-ketone structures which have a relation in equilibrium. Insoluble products which often arise *in vitro* as a consequence of oxygenation of such highly unsaturated acyl groups are assumed to be either a consequence of association which depends on the presence of a hydroxy-ketone structure or a consequence of chemical condensation in which a molecular union occurs across oxygen bridges.

This oxidative process is inhibited *in vitro* by the presence of such potent antioxygenic agents as hydroquinone and pyrogallol.<sup>5</sup> Therefore, on the assumption that similar oxygenation and condensation were responsible for the biologic transformation, an attempt was made experimentally to inhibit the transformation *in vivo* by adding hydroquinone or pyrogallol to unsaturated compounds prior to introduction into the tissues. The presence of these reagents, contrary to prediction, had no influence on the biologic transformation. Experiments of shorter duration might show retardation, but this does not seem likely in view of the data.

The addition of oxygen to unsaturated compounds such as those under discussion is greatly accelerated *in vitro* by the presence of a trace of iron or several other metals in the materials exposed to oxygen.<sup>4</sup> As a consequence, the rate of uptake of oxygen and the formation of insoluble materials are increased. On the assumption that iron would have a similar action *in vivo*, several iron salts were added to various unsaturated materials prior to injection. The results indicated clearly that, in accord with prediction, the presence of iron was effective in accelerating the biologic transformation.

The preceding results, together with previous proof that preliminary oxygenation of highly unsaturated methyl esters was required before they were convertible into membranes under biologic circumstances, encouraged a further investigation of molecular changes which are responsible for some of the morphologic findings. The experiments

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4 Coffey, S. J. *J Chem Soc London* **119** 1152 and 1408, 1921

5 Mattill, H. A. *J Biol Chem* **90** 141, 1931

which showed that the unsaturated compounds lost their initial capacity to reduce perosmic acid as they became transformed into membranes gave further impetus to a consideration of the molecular structure of the membranes

Attention was first concentrated, therefore, on demonstrable chemical alterations brought about by oxygenation of unsaturated methyl esters *in vitro*, for this treatment was required before these compounds were amenable to transformation into membranes. Two chemical alterations were demonstrated by isolation *in vitro* of crystalline phenylhydrazones and benzoyl derivatives. From such data one may conclude that hydroxyl and keto groups were formed during the period of oxygenation. This is in keeping with prediction. These data afforded a logical basis for an investigation of the insoluble products which were formed under biologic circumstances.

The presence of such groups in the mature membranes could not be proved by any direct means. The insolubility of the materials and the small amounts available for study presented major difficulties from the chemical point of view. The only alternative was to make the determinations on the materials in thin sections of tissue with the hope, first, that the chemical reagent might react at least with surface molecules and, second, that an appropriate method for detecting the presence or absence of reaction might be devised. It was a simple matter to make the tests but a more difficult one to devise a method for determining whether reaction had occurred. On the thesis that the presence or absence of reaction might be measured by an interference in the election by membranes of some stain, the resorcinol-fuchsin stain was employed. This indirect method was crude and not wholly adequate. However, I believe that this is an appropriate procedure and that within limits interpretation of the data is permissible. Better methods, however, must be devised before any considerable degree of precision can be obtained.

It is not necessary at this time to discuss the addition of the "specific" reagents, as their vagaries are not thoroughly understood. This is especially true when the compounds undergoing reaction have conjugated keto groups or keto-enol arrangements, which are distinct possibilities in the structure of the altered compounds in the tissues.

The deductions from the experimental data, subject to the preceding argument, are as follows. First, reactions between the reagents and the altered lipoids in thin sections of tissue do occur. Second, the reactions with the "keto-fixing" reagents create a state in which there is a constant decisive inhibition of the capacity of membranes to elect Weigert's resorcinol-fuchsin stain. From these data the deduction is drawn that the inhibition is a consequence of reaction with keto groups in the membranes. Third, the reactions with two reagents which characteristically condense with hydroxyl groups create a state in the

membranes by which a slight inhibition of their capacity to elect Weigert's stain is detectable. From these data the inference is drawn that reactive hydroxyl groups are present in the membranes. Fourth, that if successive determinations are made, first with a "keto-fixing" reagent and second with a "hydroxyl-fixing" reagent, the inhibitory effect produced by the "keto-fixing" reagent is reduced rather than enhanced. No explanation for this result can be given. It is contrary to prediction, but the reasons for the discrepancy may not be wholly referable to technical difficulties. These data give no cause for quantitative deductions, because the mode of action of the resorcinol-fuchsin stain is unknown and it is possible that further experimentation with other "hydroxyl-fixing" reagents will yield a more satisfactory inhibition. A real difficulty, of course, lies in the fact that even though a large number of reactive hydroxyl groups condense with a reagent, the staining reaction might not necessarily be influenced in any way. The fact that the election by elastic tissue of the resorcinol-fuchsin stain was not interfered with by any reagents except sulfuric acid adds to the complexity of the interpretations. However, it is hoped that these specific reagents, which are of great value in organic chemistry, will be of further use in histochemistry and that some of the puzzles which have arisen during this investigation of the chemical structure of these membranes will be solved.

#### SUMMARY

Previous investigations have shown that several liquid soluble unsaturated organic compounds become insoluble during a period of residence in the subcutaneous tissues of guinea pigs and that the insoluble compounds may be oriented to form continuous membranes at lipid-aqueous protoplasmic interfaces. In the studies reported here, this biologic transformation has been investigated in a sufficient number of localities to permit the hypothesis that it occurs in general in the intercellular medium.

The liquid soluble organic compounds utilized in previous investigations were not obtained directly from mammalian tissues. In this report it has been proved that compounds amenable to the transformation in question are present in ether or acetone-ether extracts of mammalian liver, blood and brain. A further naturalization of the process was achieved by demonstrating that cholesterol esters of highly unsaturated acids are susceptible to the transformation and that the insoluble products are often oriented to form continuous membranes at cholesterol ester-aqueous protoplasmic interfaces. Purified egg lecithin, on the other hand, did not yield the slightest trace of insoluble products.

Previous studies have indicated that partial biologic oxidation and subsequent polymerization of incompletely oxidized unsaturated compounds were the principal chemical mechanisms on which the trans-

formations depended. Additional support of this thesis has been obtained by investigations discussed in this paper. These studies have shown, first, that several iron-bearing compounds accelerate the transformation *in vivo*, second, that as the unsaturated compounds undergo conversion, they gradually lose their initial capacity to reduce perosmic acid, third, that preliminary oxygenation of certain highly unsaturated methyl esters is necessary before these are convertible into long continuous membranes and that an accompaniment of this treatment is the creation of hydroxyl and keto groups in some oxygenated molecules, and fourth, that these groups, especially keto groups, seem to be created in the process of membrane formation in the biologic environment.

Although the membranes are composed principally of altered unsaturated compounds, the morphologic observations here reported show clearly that protoplasm is at times implicated in the basal structure of membranes in the lipid-aqueous interfacial zone. The participation of proteins such as collagen, fibrin and marginal cytoplasm in the membrane structure may be a consequence of purely physical factors, but the data do not disprove actual chemical lipoprotein condensation.

Therefore, it is necessary to deal with long normal chain highly unsaturated acyl groups which acquire in this semibiologic system carboxyl, hydroxyl and keto polar groups on hydrolysis or partial oxidation, which associate to form insoluble long continuous membranes at lipid-aqueous protoplasmic interfaces and which merge with protoplasmic elements at such interfaces to form lipoprotein structures. These structures, as they mature, eventually lose all characteristics by which their chemical origins might conveniently be proved.

#### APPENDIX

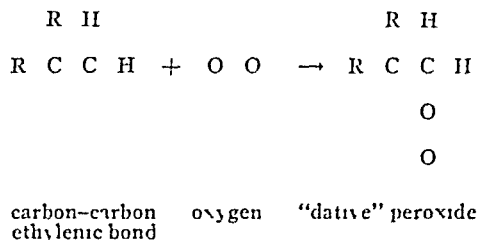
Most compounds used in these investigations belong to a general class of organic substances which undergo auto-oxidation and polymerization *in vitro*. Milas<sup>6</sup> has summarized the data of organic chemistry relevant to this subject. It may be worth while to clarify the plan and principles which underlie the present investigation by a brief discussion of the theory of auto-oxidation and polymerization of auto-oxidizable compounds.

Oxygen may exist in many states. In some states it is highly reactive and in other states relatively inert. Molecular atmospheric oxygen is a relatively inactive form of oxygen and most compounds are indifferent to its presence. Other compounds, classified in general as auto-oxidizable compounds, will readily react with this form of oxygen. In the compounds used in the present experiments the ethylenic linkages in the long carbon chains of acids or acyl groups are the important reactive centers. One molecule of oxygen may add to each of the reactive double bonds. The oxygenated compound thus becomes a "dative" peroxide. The oxygen in this compound exists in a highly reactive state and

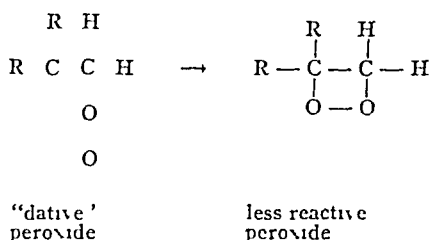
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6 Milas, N. A. Chem. Rev. **10** 295, 1932.

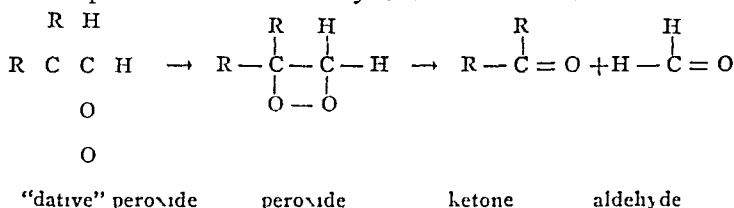
may enter into reactions which were not possible prior to its addition to an ethylenic linkage. A "dative" peroxide may be represented electronically as follows:



This compound as a rule exists only in theory. In general it is unstable and represents the highest level of oxygen "activation." The reaction just illustrated may be reversible or irreversible. If reversible, the oxygen is released and the carbon-carbon ethylenic bond is regenerated, becoming again susceptible to oxygenation. If the reaction is irreversible, the "dative" peroxide may undergo one of several changes. It may undergo rearrangement to form a relatively stable less reactive peroxide, which may be represented as follows:

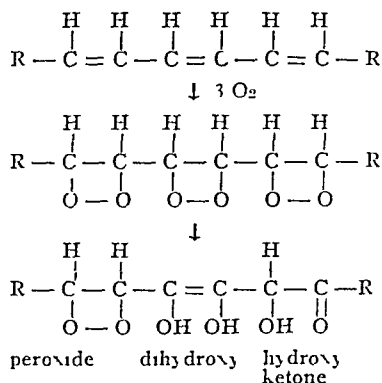


It may decompose to form an aldehyde and a ketone as follows:



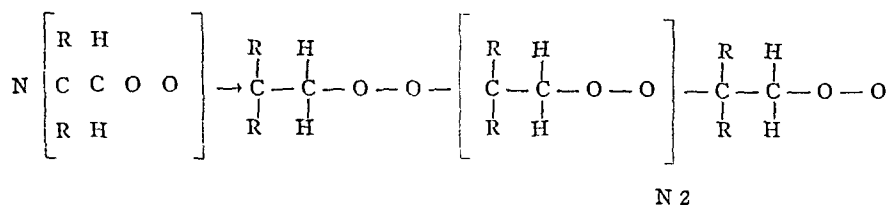
The aldehyde and ketone are in turn available for further oxidation.

When dealing with ethylenic linkages in a chain of carbon atoms, a possible sequence, which is supported by experimental observations, may be given as follows (the hypothetical "dative" peroxide is omitted from the formulation):

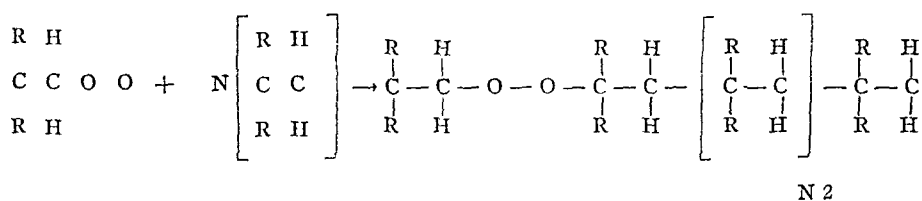


This illustrates the possibility of keto-enol tautomeric rearrangements within single molecules and the creation of reactive polar groups along hydrocarbon chains.

The polymerization of long chain highly unsaturated auto-oxidants is a complex process and is not well understood. There is some evidence, however, which indicates that "dative" peroxides may polymerize with similar peroxides to form compounds of high molecular weight. The schematic representation of this process is as follows:



The "dative" peroxide may polymerize with other auto-oxidant molecules which have not added oxygen. In this instance a chain reaction may occur in which polymerization of the unoxygenated auto-oxidant is the only significant event. This may be shown by the following formula:



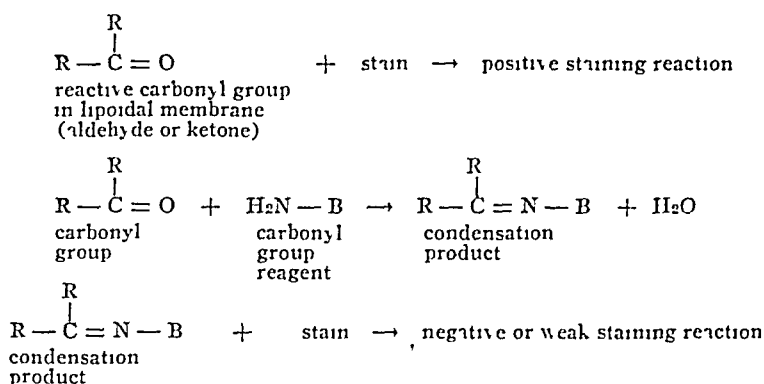
In the course of the study of the behavior of complex auto-oxidant molecules in the tissues of animals where little free oxygen is supposed to exist, the thesis is held that at least some similarities exist between the behavior of these auto-oxidants *in vitro* and *in vivo*. In general there can be little doubt, in view of the evidence, that polymerization of auto-oxidant molecules progresses smoothly in the vital system under investigation. The process is more rapid in the biologic system than under uncatalyzed conditions of oxygenation within the biologic temperature range *in vitro*. It seems logical to deduce that some biologic mechanisms are responsible for catalyzing the process in the tissues and for transporting oxygen to the lipoidal substrate.

At first glance, it would seem a hopeless task to inquire into the more minute structure of the interesting membranes which form at the lipid-aqueous protoplasmic interfaces. If any information is to be gained, microhistochemical methods are required.

It has been established by previous workers that the black staining of lipoidal compounds by perosmic acid is referable to the presence of double bonds in the long carbon chains of the lipoids. The black staining is attributed to the impregnation of the lipoids with black reduction compounds of perosmic acid. This histochemical reaction therefore becomes of use in these studies, for it illustrates that lipoidal compounds under the conditions of these experiments may gradually lose an initial capacity to reduce perosmic acid. This evidence indicates that at least a majority of the ethylenic linkages have disappeared, presumably in part by reaction with oxygen.

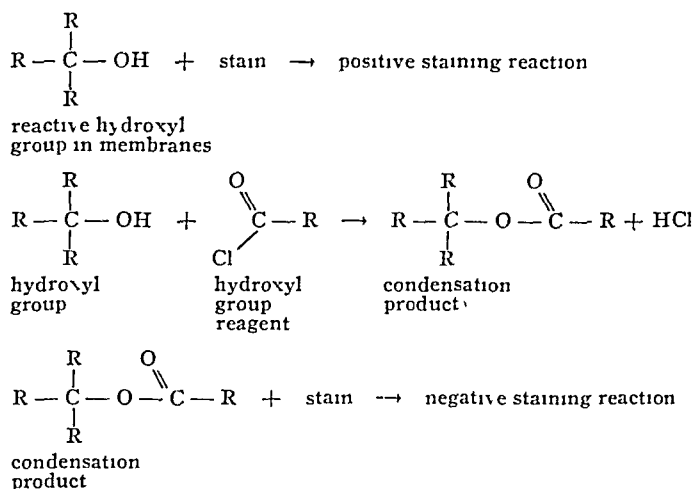
The proof that oxygen is implicated in satisfying available ethylenic linkages during the biologic transformation of lipoidal compounds into membranes must necessarily rest on a satisfactory demonstration of oxygen which is bound in the lipoidal membranes. Functional oxygen-containing groups, such as peroxide groups, carbonyl groups and hydroxyl groups, represent reactive centers for study, if the formulation given is logically followed.

For investigating the possible presence of available carbonyl groups which will react with carbonyl group organic reagents, the following scheme was devised for use on thin sections of tissue



An appropriate stain which was useful as a detector of this reaction proved to be Weigert's resorcinol-fuchsin stain. In other words, membranes which normally elect the resorcinol-fuchsin stain fail to elect this stain if they have been previously treated with reagents which characteristically condense with reactive carbonyl groups. This is presumptive evidence that some compounds in the membranes possess reactive carbonyl groups and that these groups may be primarily responsible in this instance for the positive staining of the membranes by the resorcinol-fuchsin method.

A similar scheme was devised for demonstrating reactive hydroxyl groups in the lipoidal membranes. This may be formulated as follows:



Thus far, attempts to demonstrate reactive hydroxyl groups in compounds of which the membranes are composed have not been very successful. This may be due to one of several factors. First, reactive hydroxyl groups, if present, may not be available in sufficient numbers. Second, the reagents may not react with the groups which are present. Third, an appropriate staining reaction which depends on the presence of reactive hydroxyl groups has not been used. The third possibility is most likely, and a further study of this problem is indicated. Other projects include a search for peroxide and keto-enol groups. Finally, the problem of possible actual chemical conjugation of tissue proteins with the altered lipoids adds further complications to the study. This problem must be attacked primarily by synthetic methods, controlled by histologic observations.

# TRANSPLANTATION OF TOOTH GERM ELEMENTS TO MARROW CAVITIES OF TIBIAS OF KITTENS

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AND

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The fact that occasionally a tumor resembling an adamantinoma develops in the human tibia prompted this study of autogenous tooth germ elements transplanted into the marrow cavities of tibiae of kittens. We were particularly interested in the immediate histologic changes taking place in the transplants <sup>1</sup>

Six kittens, each weighing about 1 Kg, were anesthetized with sodium amytal. An unerupted canine tooth was exposed by excision of a thin plate of jawbone and removed. The calcified dentin and enamel were discarded. The soft tissue of the tooth was placed in warm Ringer's solution. The membranes were halved longitudinally, one half was discarded and the other placed in the upper third of the tibial marrow cavity.

The site for reception of the transplant was prepared as follows. The periosteum on the medial surface of the tibia was retracted. Several small drill holes were made in the cortex, outlining a small rectangle of bone. This rectangular shell was removed, a small amount of marrow substance was curetted, and the tooth membranes were placed in the resulting cavity. The periosteum was then sutured so that the cortical defect was covered over.

In some of the animals a similar procedure was pursued at a later date with the opposite unerupted canine tooth and the opposite tibia. In several cases one of the legs containing a transplant was amputated after a short interval to permit study of the early changes.

Individual transplants were left in situ for three, twenty-one, thirty-one, thirty-one, fifty-one, one hundred and thirty-one, one hundred and sixty-two, one hundred and sixty-six and one hundred and eighty days, respectively and then removed for examination.

After a three day period, most of the pulp cells were found flat, some were tricornuate in general shape and had fine terminal filaments. At the periphery of the pulp mass there were thin sheets of nonactive

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From the Laboratory Division of the Hospital for Joint Diseases

1 Wolfert, B, and Sloane, D. J Bone & Joint Surg 20 1011, 1938





Fig 1—Photomicrograph of transplanted dental pulp after a three day interval,  $\times 100$  Note the viable pulp cells as well as the areas of necrosis at the margin. At the extreme periphery is a thin layer of epithelial cells, most probably ameloblasts



Fig 2—Photomicrograph of a transplant after a twenty-one day interval,  $\times 100$  Note the active islands of dentin formation

ameloblasts. No evidence of active formation of dentin or of enamel was noted (fig 1)

In another transplant, examined twenty-one days after implantation, the active formation of dentin was the most striking feature (fig 2). The pulp cells had hypertrophied, were lined up in a regular fashion and adjoined the predentin zone. Interspersed among the fibrils of the pulp matrix were numerous amorphous calcaeous masses, apparently

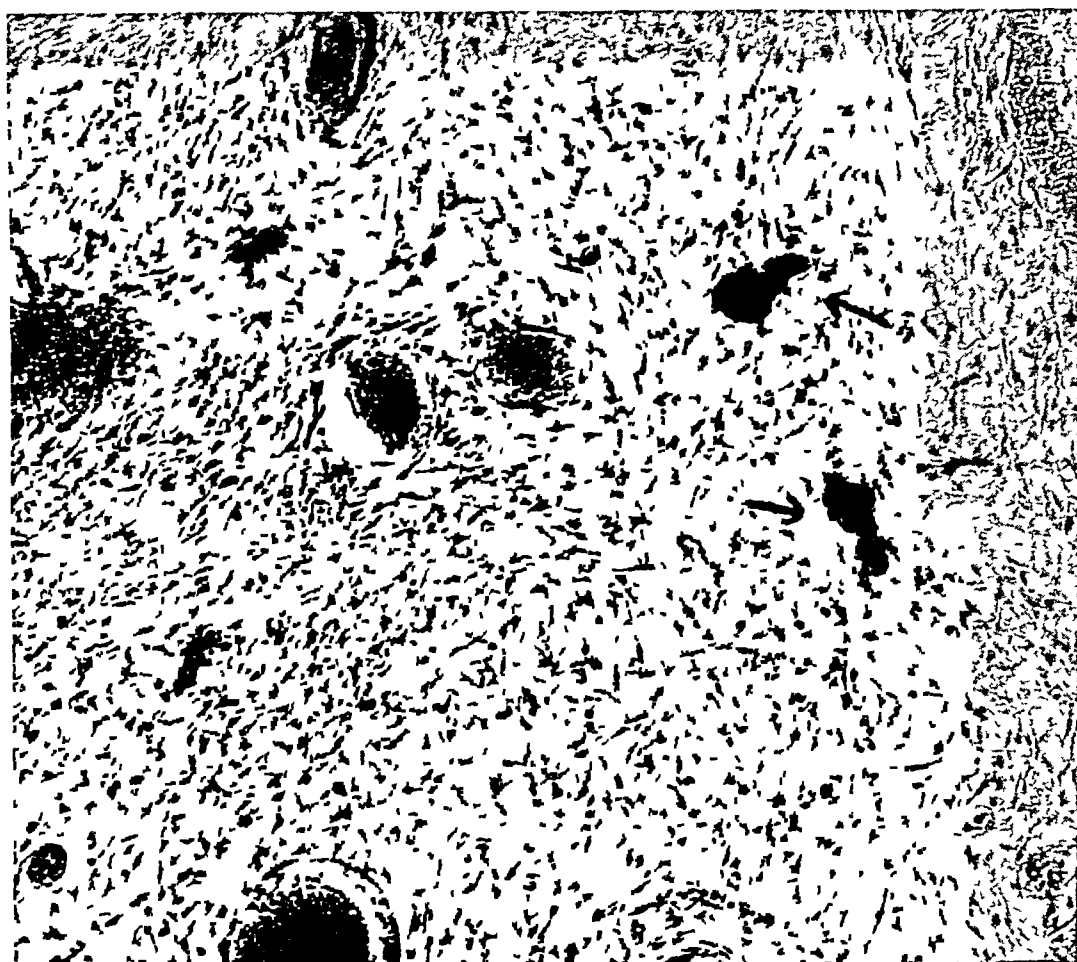


Fig 3—Photomicrograph of a twenty-one day transplant,  $\times 100$ . The living pulp shows several amorphous calcospherites (arrows)

similar to the calcospherites found in normal dentin (fig 3). No new enamel or islands of ameloblasts were noted.

After thirty-one days, a considerable amount of dentin was present. A good portion of it was acalcaeous. In other parts of the transplant, small and large segments of Hertwig's membrane were encountered. Differentiation could be seen to have been progressing. In still other



Fig 4—Photomicrograph of a thirty-one day transplant, revealing activity of Hertwig's membrane in the pulp tissue,  $\times 50$  Note the island of ameloblasts lying outside the pulp tissue (arrow) and showing no enamel formation

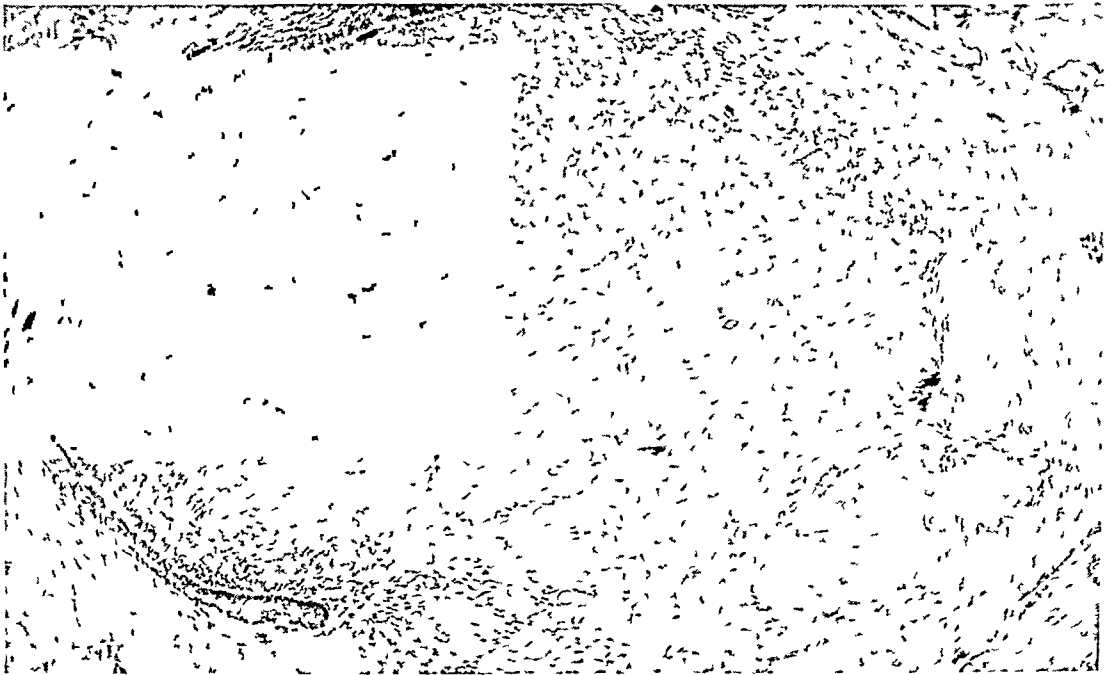


Fig 5—Photomicrograph of a thirty-one day transplant, showing a large segment of Hertwig's membrane with early differentiation,  $\times 65$  Note the cellular proliferation of the pulp near the membrane

portions of the transplant there were islands consisting of aggregate masses of epithelial cells, there were nests of ameloblasts (fig 4)

In another transplant examined after thirty-one days, several large islands of epithelium were observed. Each presents definite evidence of an early attempt at formation of both enamel and dentin. It is of special importance that where the islands of epithelium were not in contact with pulp there were no signs of enamel formation (figs 4, 5 and 6)



Fig 6—Photomicrograph of a thirty-one day transplant showing two islands of proliferating ameloblasts in the pulp,  $\times 100$ . Note that the contiguous pulp cells are being transformed into odontoblasts

When new bone was being formed near the transplanted dental pulp the osteoblasts were unusually large, some showing mitosis and others forming giant cells (fig 7)

Nothing very unusual was found in the tibia after the thirty-one day period. Dentin formation had continued, and most of the pulp

had been crowded out by the newly formed bone. One preparation (fig 8) revealed a large area of osteodentin in process of formation.

#### COMMENT

This study shows in the first place that autogenous tooth germ elements transplanted into the tibial marrow can regenerate. Hertwig's



Fig 7—Photomicrograph of a thirty-one day transplant showing new bone formation near active Hertwig's membrane,  $\times 85$ . Note the unusually large dark staining osteoblasts (arrow).

sheath survived and proliferated in its new environment. Special interest is attached to the finding that when the enamel and odontoblast layers were in proximity both showed signs of continued activity. In places

where ameloblasts were absent the transplanted odontoblasts did not show continued activity, merely forming irregular sheets of dentin. Furthermore, the pulp cells did not differentiate into odontoblasts when ameloblasts were not present nearby. On the other hand, where ameloblasts were present without adjoining pulp cells or odontoblasts, they merely became transformed into islands of epithelial tissue.<sup>2</sup>



Fig 8—Photomicrograph of a one hundred and sixty-two day transplant showing a large mass of osteodentin,  $\times 45$ . Note zone of predentin with a layer of odontoblasts.

If these observations are applicable at all in the interpretation of the so-called adamantinoma of the human tibia, they suggest that this tumor may be an ameloblastoma rather than an odontoblastoma (adamantinoma). If this tumor thus grows out of misplaced tooth germ, the

<sup>2</sup> Legros, C, and Magitot, E. *Compt rend Acad d sc* **78** 357, 1874.  
Huggins, C B, McCarroll, H R and Dahlberg, A A. *J Exper Med* **60** 199, 1934.  
Glasstone S. *J Anat* **70** 260, 1936.

observed lack of formation of enamel or of dentin in connection with it would be due to the absence of odontoblasts or pulp cells adjacent to the ameloblasts<sup>3</sup>

#### SUMMARY

Tooth germ elements of kittens regenerate after autogenous transplantation into the tibial marrow cavity. Which of the various germ elements will proliferate and to what extent depend on the degree of preservation of the enamel-dentin relationship.

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3 Robinson, H B G Arch Path **23** 664 and 831, 1937

# SOLID TO CYSTIC DEGENERATION IN AN AMELOBLASTOMA

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From histologic studies Kronfeld<sup>1</sup> concluded that "the cystic adamantinoma develops from the solid type by cystic degeneration of the stellate reticulum" Robinson,<sup>2</sup> commenting on a histologic study of 4 cases, said, "While no single ameloblastoma has yet been traced from solid to cystic form through histologic studies, it seems highly probable that such a transition takes place While this evidence is highly suggestive, it cannot be claimed that such a process is known to take place" This opinion is confirmed by a review of the literature<sup>3</sup>

This histologic evidence seems to indicate that the ameloblastoma begins as a homologue of the developing tooth and follows the general structural development seen in the stages of odontogenesis up to the point of function (laying down of enamel) From that point on the neoplasm follows a degenerative course leading to the formation of a multicystic tumor The degeneration appears to occur in the stellate reticulum-like cells in the center of the follicle This mode of transition leaves the ameloblastoid columnar cells as a cystic lining, although these cells may be compressed to a cuboid or squamous form<sup>2</sup>

In the previous histologic study of ameloblastoma<sup>2</sup> a solid type was described as resembling basal cell carcinoma, and a photomicrograph of it was used as an illustration of early cystic change in the follicles After the publication of that report the patient returned with a huge tumor of the mandible The case history follows

A man 49 years of age, married, said that about one year prior to examination he noticed a hard painless nodule, on the buccal surface of the right mandibular ridge It grew slowly until about two months before presentation, when it sud-

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This work was supported in part by grants from the Carnegie Corporation of New York and from the John and Marv Markle Foundation

1 Kronfeld, R J Am Dent A **17** 681, 1930

2 Robinson, H B G Arch Path **23** 664, 1937

3 Robinson, H B G Arch Path **23** 831, 1937



denly became swollen and painful. Two days later a bloody discharge was noticed about the mandibular right second molar tooth. This disturbance was repeated in about two weeks' time. The mandibular right second molar tooth was extracted. On examination the growth appeared as a nontender, painless hard nodule. A biopsy was made, and the growth was reported as ameloblastoma. At operation the cyst was worked free from the bone, care being taken to preserve the structure of the bone. The tumor mass was split and delivered in small pieces. The area was curetted and the inferior dental artery ligated. The cavity was cauterized with phenol and washed with alcohol.

The gross specimen consisted of several small pieces of tissue of irregular shape, with fragments of adhering bone. The cut surface was smooth, glistening, dense and white, with several smooth red areas, which probably represented cysts.



Fig 1—Mass removed at operation. The size of the tumor mass with the attached bone was 8 by 6 by 6 cm.

Microscopically, columns of epithelium were found in connective tissue stroma. The epithelium was of columnar type, with a delicate basement membrane and more irregular epithelial cells between. In places the intercolumnar cells suggested stellate reticulum, and in still other areas small cysts containing a homogeneous pink-staining material were found. While epithelial pearls were not seen, the appearance of some of the cysts suggested a tendency toward keratinization. The epithelium of the neoplasm showed connection with the oral mucous membrane.<sup>2</sup>

The patient failed to return for further study and possible partial resection, as ordered. Five years after the last report he presented a huge swelling of the right mandible and submaxillary area. Roentgenographically, destruction of the

bone was demonstrated in areas extending from the symphysis to the angle. The mass was not painful, it was slightly fluctuant and suggested lobulation. At operation the neoplasm and mandible were exposed through an external incision, and the bone was sectioned just beyond the symphysis on the left and posterior to the angle on the right.<sup>4</sup> The removed mass is seen in figure 1.

Sections were made through the entire tumor mass and the attached mandibular bone. Figure 2 shows one of these sections. Cysts may be seen in it, ranging from microscopic size to 4 by 4.5 cm. The cysts are lined by epithelial cells of cuboid, columnar and squamous type, and the centers of the cysts are occupied by mucoid material in droplets and solid sheets with occasional cholesterol slits. In some areas the bone has disappeared, while in others evidence of growth pressure is evidenced by osteoclastic activity on the cyst side and osteoblastic activity on the other. There are large areas in which solid strands and columns of

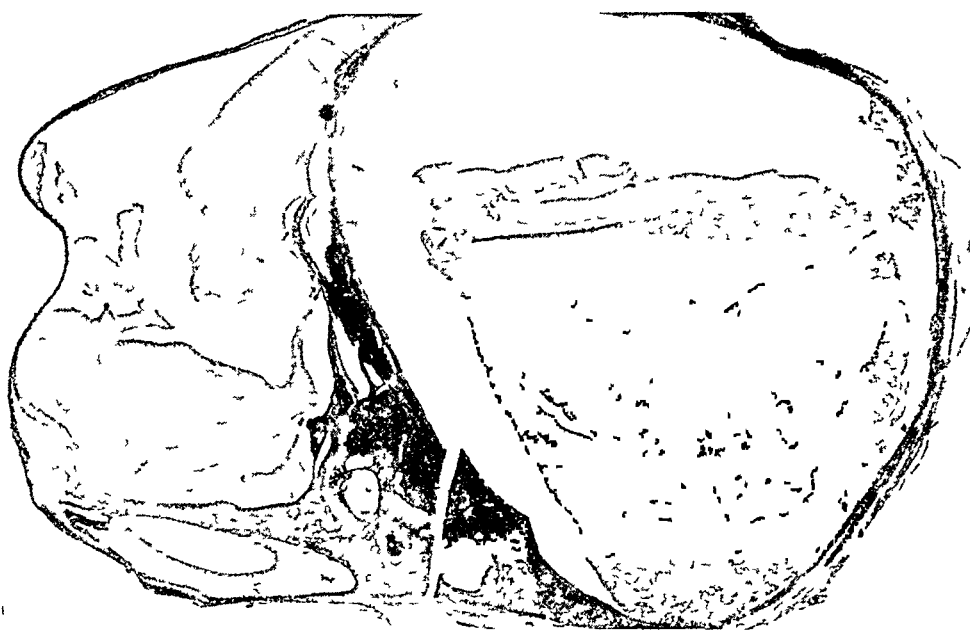


Fig 2—Photomicrograph of a cross section of the entire mass, showing a cystic area and bone,  $\times 25$ . Note the large and small cysts, the areas of bone and the solid epithelial strands and mucoid-like material in the cyst cavities.

epithelial cells remain. These resemble the areas seen at the previous operation,<sup>5</sup> with columnar cells externally and more irregular or stellate reticulum-like cells centrally (fig 3A). The epithelium lining the cysts, although it may be compressed to squamous form, is still capable of differentiation and proliferation. In figure 3B an area from the cyst wall is seen with proliferation of the epithelium and differentiation toward the form resembling the dental lamina. Early cystic change is seen even in this epithelium. In the same area, adjacent to this active proliferation of epithelium, osteoclastic activity is seen. The stroma, not the epithelium, is rich in blood vessels.

4 The operation was done by Forrest Young and W. R. J. Wallace.

5 Robinson,<sup>2</sup> figure 2A.



Fig 3—*A*, area from figure 2 at higher magnification, showing epithelial strands with columnar cells externally and stellate cells centrally. Note early cystic degeneration. *B*, area from figure 2 at higher magnification, showing the proliferative power of the cyst lining. Note the resemblance of the epithelial strand to dental lamina, the intraepithelial cyst formation and the osteoclastic activity at bone adjacent to the area of epithelial growth.

## COMMENT

The solid to cystic change in the ameloblastoma previously demonstrated in series of cases was not corroborated by clinical studies or by a microscopic study of these changes in a single case. The present case would not be of great value as an isolated example, but coupled with the laboratory series it can be presented as strongly confirming the previous indications. It is rather interesting that the solid phase of this identical neoplasm was used as an example of a solid ameloblastoma showing an early cystic tendency.

The demonstration that the ameloblastoma may degenerate from solid to cystic form should be of value to the surgeon. If the tumor mass can be removed in its solid stage, enucleation and cauterization may suffice, for it will not yet have extended its follicular growth between the bone trabeculae. At a later date the cystic transition may have begun, and the many strands and cysts will be growing by pressure into the bone spaces. At this stage enucleation is difficult or impossible, and resection becomes the operation of choice. Ivy and Curtis<sup>6</sup> recommended primary resection probably because the surgeon seldom sees the ameloblastoma in its early painless and slow-growing stage. The responsibility falls on the clinician, who must make the diagnosis early.

## SUMMARY

A case of multicystic ameloblastoma is presented. Five years previously a growth of this neoplasm was removed and was a solid tumor. This change is strong confirmation of previous laboratory demonstrations that the ameloblastoma changes from a solid to a cystic stage through degeneration of the stellate, reticulum-like cells.

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6 Ivy, R. H., and Curtis, L. *Ann Surg* **105** 125, 1937

# ATTEMPTS TO PROPAGATE FOWL TUMORS PRODUCED BY BENZPYRENE AND BY VIRUS

SITES OF IMPLANTATION USED EYE OF CHICKEN AND CHORIO-ALLANTOIC MEMBRANE OF CHICK EMBRYO

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AND

JOHN R HERMAN

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A transmissible spontaneous fowl tumor was described by Rous<sup>1</sup> (1910), and in the following year he showed that it could be transmitted with either cellular material or cell-free filtrate<sup>2</sup> Rous and Murphy<sup>3</sup> propagated this tumor by introducing a cell-free filtrate onto the chorioallantoic membrane of the chick embryo Subsequently several different "filtrable tumors" were described by other investigators Since chicken tumors can be produced readily by application of tar, the question arises whether or not the tumors induced by a chemical contain a virus

Murphy and Landsteiner<sup>4</sup> were the first to investigate this problem They produced spindle cell sarcoma in 2 chickens by injections of coal tar One of the tumors was transmitted by cellular material for 11 generations and proved to be highly invasive These investigators were unable at any time to transmit the sarcoma with a cell-free filtrate or a desiccate Peacock<sup>5</sup> induced fowl sarcoma by injections of tar and of 1,2,5,6-dibenzanthracene-lard mixtures and likewise failed to transmit the tumors with cell-free material McIntosh<sup>6</sup> produced sarcoma by injecting tar, and, contrary to Murphy and Landsteiner, and Peacock, was able to transmit the tumors with filtrates Because McIntosh was studying the virus of Rous sarcoma at the same time, Peacock thought that this virus might have spread among the birds that were used in the study of chemically induced tumors

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This study has been aided by grants from the Anna Fuller Fund, the Jane Coffin Childs Memorial Fund, the John and Mary R Markle Foundation and the Ophthalmological Foundation, Inc

1 Rous, P J Exper Med **12** 696, 1910

2 Rous, P J Exper Med **13** 397, 1911

3 Rous, P, and Murphy, J B J A M A **56** 741, 1911

4 Murphy, J B, and Landsteiner, K J Exper Med **41** 807, 1925

5 Peacock, P R J Path & Bact **36** 141, 1933

6 McIntosh, J Brit J Exper Path **14** 422, 1933

McIntosh also described leukemia in the birds that received tar. From his data it is not possible to determine whether the diseases observed in the blood-forming organs were due to leukemia or a leukemoid reaction, were produced by the agent of sarcoma, occurred independently or were a secondary reaction to sarcoma. Mellanby<sup>7</sup> inoculated chickens with Rous sarcoma and dibenzanthracene-induced tumor at the same time. The filtrate of the latter gave rise to a Rous sarcoma, whereas the cellular material produced a growth similar to the original chemical tumor with none of the characteristics of the Rous sarcoma. This experiment indicates that in fowl the filtrable agent of Rous sarcoma can pass into a chemically induced tumor which itself has no filtrable agent.

In view of the confusion existing in this field, it seemed desirable to reinvestigate the problem, and find out, if possible, whether tumors induced by a chemical contain a filtrable virus. In the experiments which will now be described, we attempted to propagate fowl tumors induced by benzpyrene in chicken eyes and breasts and on the chorioallantoic membranes of chick embryos. Tumors which were highly malignant to their hosts were produced in chickens with benzpyrene. However, they could be passed to only a few of the many birds that were inoculated and in this respect differed from the readily transmissible virus tumors.

Since attempts to propagate these benzpyrene-induced tumors by inoculation into the anterior chambers of chicken eyes and on the chorioallantoic membranes of chick embryos were unsuccessful, it was necessary to control the experiment by propagating one of our readily transmissible sarcomas by the same procedures.

#### MATERIAL AND METHODS

Young Rhode Island Red and Barred Plymouth Rock chickens weighing about 500 Gm were used in the transmission experiments.

The method used by us for implanting material on the chorioallantois of the chick embryo has been described in detail by Burnet.<sup>8</sup> Fertile eggs from Rhode Island Red hens were incubated for ten days at 37.5 C and turned daily. They were then candled to locate the air sac and the embryo, which were outlined with a wax pencil. By the use of an electric rotary tool with a carborundum disk attached to the shaft, a triangular area, 1 cm. to a side, was cut through the shell over the outlined embryo without penetrating the adherent underlying shell membrane. The upper half of the egg was cleansed with 70 per cent alcohol, and a thin layer of melted paraffin was placed over the triangular cut. A small

7 Mellanby, E. J. Path & Bact. **46** 447, 1938.

8 Burnet, F. M. The Use of the Developing Egg in Virus Research, Medical Research Council, Special Report Series, no 220, London, His Majesty's Stationery Office, 1936.

opening 2 to 3 mm in size was made over the air sac with the rotary tool and likewise coated with paraffin. The triangle of shell was lifted off, and the air sac punctured with a sharp instrument. By creating suction with a rubber tube opening into the air sac, the chorioallantois was separated from the shell membrane, which was then removed under sterile precautions and the inoculum placed over the chorioallantois. The triangle was rimmed with petiolatum and a cover slip placed over it. The egg was returned to the incubator for a period of ten days. Then the upper part of the shell was removed, and the chorioallantois collected in Tyrode's solution. Part of the membrane was placed in Zenker's fixative, and part was cut into 1 mm particles for further passage. The collected material was always cultured prior to further implantation on egg membranes.

The following technic was used for the transplantation of tumor particles into the anterior chambers of chicken eyes. The eye was first anesthetized with 2 per cent butyn solution, and the eyelids were separated with a wire speculum. A 3 mm incision was made at the corneoscleral junction with an angle keratome. The tumor implant was grasped with a curved iris forceps and was inserted through the incision into the anterior chamber. The tumor particle was observed at frequent intervals in order to determine the increase in size.

#### PRODUCTION OF BENZOPYRENE TUMORS IN CHICKENS

Six chickens were each given 2 intramuscular injections of 10 and 5 mg of benzpyrene in lard at the same site at an interval of twenty-eight days. Two months later 3 of these chickens had palpable tumors at the sites of inoculation. A fourth chicken died after three months without having had a detectable tumor during life, but at autopsy a tumor measuring 3 by 2 by 2 cm was found, which proved to be fibrosarcoma. Transmission of this growth was not attempted. The 2 remaining chickens, killed ten months after inoculation, had no tumor.

The chickens with benzpyrene-induced tumors that were studied experimentally will be designated as chickens 1, 2 and 3. Biopsy specimens were removed from these tumor-bearing chickens, and part of each tissue specimen removed was fixed for microscopic study. Another part was set up in tissue cultures, while the remaining part was dried and frozen.

*Chicken 1*—A biopsy was made of a tumor measuring 2 cm in the greatest diameter eighty-five days after the first injection. A section of this tumor showed fibrosarcoma (fig 1A). The chicken died six months after the initial injection of benzpyrene. At autopsy 2 tumors were found. One was in the breast and the other in the thigh. Each measured about 4 cm in the greatest diameter. These tumors were firm, cut with increased resistance and showed a tendency toward nodular formation. Soft yellow areas that measured from 2 to 4 mm across were noted in the central parts. The liver was found to be moderately enlarged and contained irregularly scattered discrete gray-white nodules measuring 2 mm in diameter. Microscopically the portal spaces showed

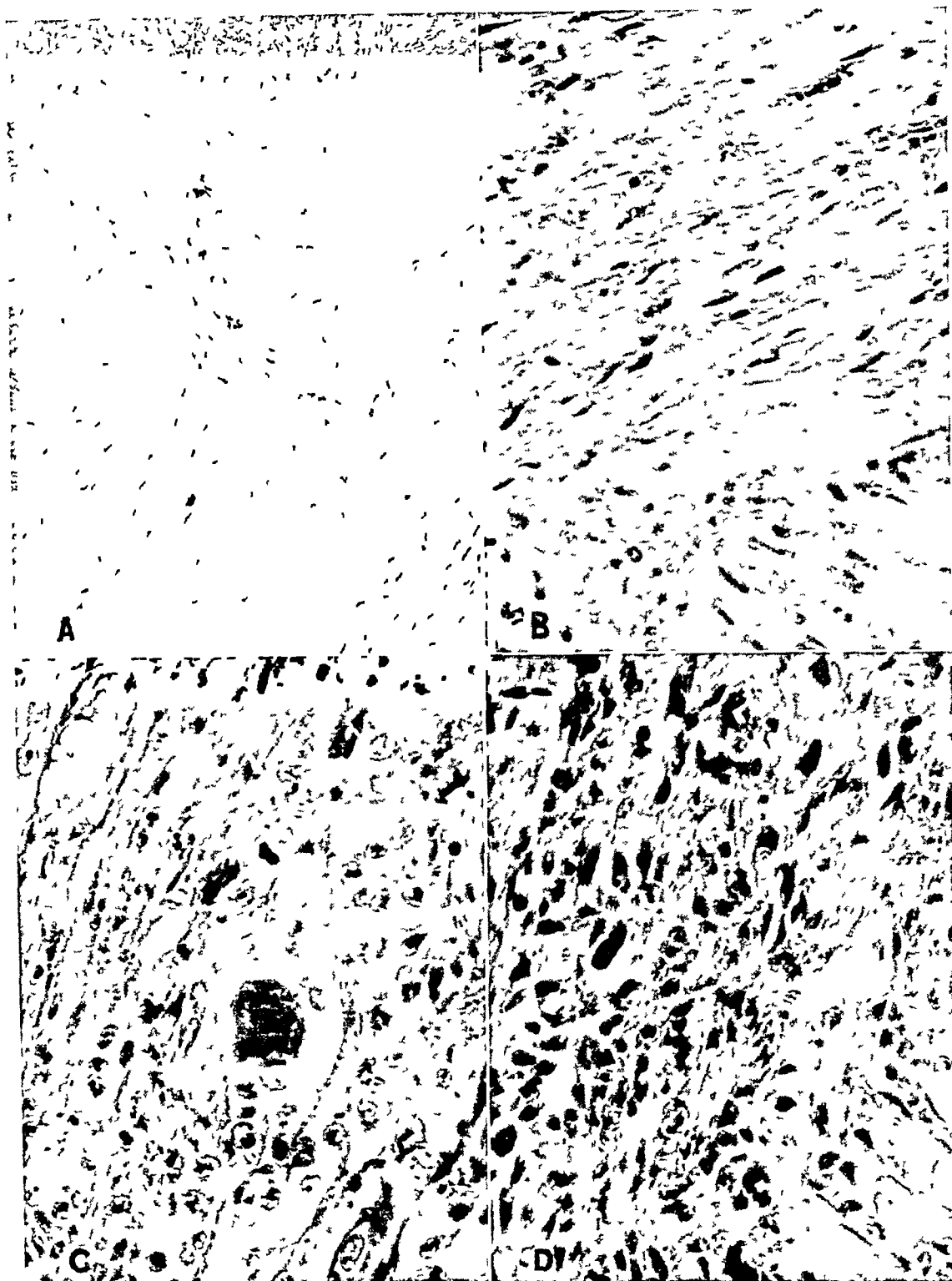


Fig 1—*A*, low power photomicrograph of the benzpyrene tumor from chicken 1 to illustrate the general arrangement of the tumor cells in whorls,  $\times 39$  *B*, photomicrograph of the tumor from chicken 2,  $\times 357$  The tumor cells are elongated, vary in size and resemble fibroblasts Many nuclei show chromatinolysis *C*, photomicrograph of the tumor from chicken 3,  $\times 357$  The cells are large and irregular in size and shape The nuclei are either vesicular or pyknotic Few mitotic figures are seen There is a large multinucleated giant cell in the field *D*, section from the transplanted tumor of chicken 3 a,  $\times 357$  The tumor cells are irregular in shape, size and arrangement, and the nuclei are pyknotic and vary in size A few muscle fibers are seen



extensive infiltrations of myelocytes. The spleen was twice the normal size and mottled with many round white areas, sections of which showed masses of myelocytes. There were many similar cells in the splenic pulp and capillaries. This chicken had myelocytic leukemia associated with spindle cell sarcoma.

*Chicken 2*—A biopsy was performed three months after injection of benzpyrene on a tumor measuring 3 by 2 by 2.5 cm. The section of this tumor reproduced in figure 1 *B* shows sarcoma. The bird was killed nine months after the initial injection. A tumor measuring 10 by 8 by 6 cm. was found in the right breast and a smaller tumor in the left breast. These tumors were firm and cut with increased resistance, soft areas of necrosis that measured 2 to 6 mm. across were noted in the central parts. The lungs contained a few small metastatic nodules, situated beneath the pleura, measuring 5 mm. across. The liver was slightly enlarged and contained minute raised grayish nodules, micro-

TABLE 1—*Transplantation Experiments with Benzpyrene-Induced Tumors*

Tumor	Site of Injection										
	Intramuscular Site			Anterior Chamber			Intravenous Site			Chorioallantois	
	Chicks	Injections	Chicks in Which Tumors Developed	Chicks	Injections	Chicks in Which Tumors Developed	Chicks	Injections	Chicks in Which Tumors Developed	Eggs	Eggs in Which Growth Developed
1	24	26	0	4	4	0	4	4	0		
2	15	20	0	6	6	0				144	0
3	47	99	1	23	34	0	5	5	0		

scopic examination showed these to be infiltrations of small lymphocytes. The spleen was slightly enlarged but was normal on microscopic examination.

*Chicken 3*—A small tumor which was palpated at the site of injection of benzpyrene after 2 months became progressively larger and at the end of five months measured 4 cm. in the greatest diameter. At this time a biopsy was made, and a section of the growth (sarcoma) is reproduced in figure 1 *C*. This bird lived for two months after biopsy. At autopsy it was emaciated, and in the left breast there was a large necrotic tumor measuring 7 by 6 by 5 cm. There were small tumor nodules in the heart, lungs, liver and kidneys, all of which proved to be sarcoma.

Eighty-six chickens (table 1) received a total of 208 injections of fragments of the three benzpyrene-induced tumors. There were 155 intramuscular and 9 intravenous injections. As the figures indicate many chickens received multiple injections. Only 1 (3 a) of 10 chickens receiving tumor fragments from chicken 3 showed a growth. This tumor, illustrated in figure 1 *D*, was noted forty-six days after the injection.

tion and at that time measured 1.5 cm in its greatest diameter. A second subpassage was made into 13 chickens, and only in 1 (3b) did small tumors develop that were recognizable at autopsy. No other passage of these tumors was attempted.

#### ATTEMPTS TO GROW THE BENZPYRENE-INDUCED TUMOR ON THE CHORIOALLANTOIS AND IN THE CHICKEN'S EYE

Woodruff and Goodpasture<sup>9</sup> showed that the chorioallantois is a favorable site for the study of virus infections. Since Rous sarcoma can be grown by introducing either a filtrate or cellular material onto the egg membrane, an attempt was made to propagate a benzpyrene-induced tumor by this method in order to determine whether a virus was associated with it. Two of the chickens with benzpyrene-induced tumors had died before we commenced work with the egg membranes, and only chicken 2 was available for these studies. The attempt to grow this tumor on the chorioallantoic membrane was unsuccessful.

Fragments of this growth were implanted on 8 egg membranes 11 days old, in one of which a nodule developed, measuring 4 by 2 by 2 mm, after ten days' incubation. However, histologic examination of this growth proved that it was a proliferation of the mesoderm and not a tumor. The cells were compact and very regular in cytologic detail, they contained few mitotic figures. They did not bear any resemblance to the cells of the original tumor. Nevertheless, small fragments of this nodule were placed on 8 more membranes, but no tumor was produced. Another subpassage was performed, and this also gave negative results. The implantation did not kill any of the developing embryos. The nodule grown on the egg membrane was inoculated into the breasts of 4 chickens, which remained free from tumors after five months.

In all, 144 inoculations on the chorioallantoic membrane were done, and none was successful (table 1).

Transplants from the tumors of chickens 1, 2 and 3 were placed in the anterior chambers of chickens' eyes in 44 instances. They did not increase in size during a period of four months, and in most cases they became smaller.

It was conceivable that the benzpyrene tumors failed to grow because the experimental birds were not inbred. In an attempt to adapt the sarcoma cells to alien plasma, material from different biopsy specimens of the benzpyrene-induced tumors was first grown in tissue cultures. A total of 26 chickens was inoculated with these cultures, none with success. Four series of specimens of the same material were carried to three successive passages on the chorioallantois but were also unsuccessful.

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9 Woodruff, A. M., and Goodpasture, E. W. *Am J Path* 7:209, 1931.

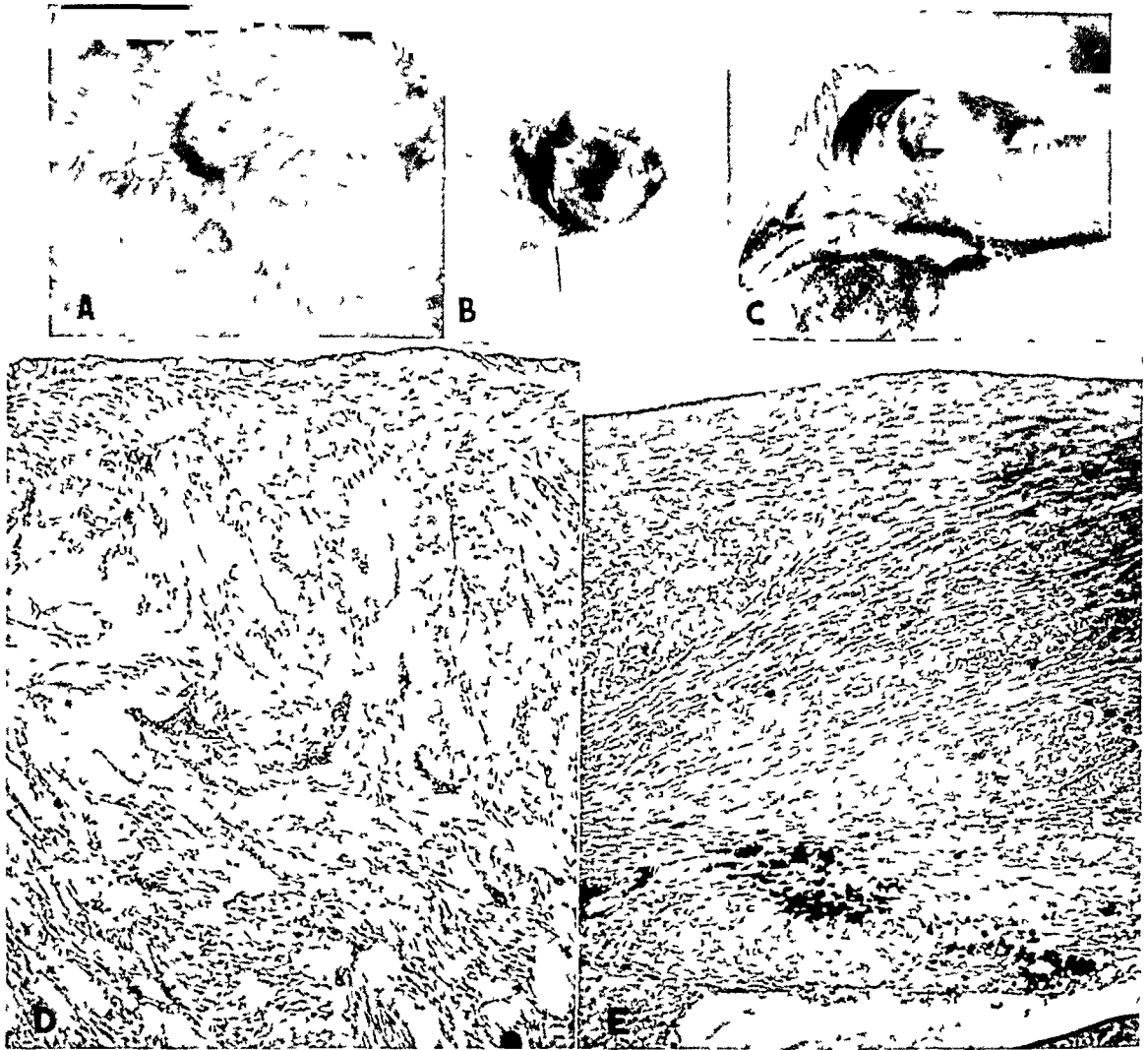


Fig 2—*A*, tumor measuring 11 by 9 by 8 mm, produced in ten days on the chorioallantois of the chick embryo by implantation of a fragment of sarcoma 13,  $\times 103$  *B*, higher magnification of the tumor illustrated in *A* The chorioallantois has been removed, and a discrete growth is seen on the inner aspect of the membrane *C*, photograph to show the eye of a chicken twenty-seven days after implantation of sarcoma 13 in the anterior chamber The palpebral fissure is wide because of increased intraocular pressure The pupil and iris are obscured by gray opaque tumor tissue *D*, low power photomicrograph showing a layer of ectodermal cells of the chorioallantois,  $\times 79$  Some of these are degenerated Beneath this layer and involving only the mesoderm is a diffuse infiltration of tumor cells, which are spindle shaped and loosely connected Few mitotic figures are seen *E*, photomicrograph showing diffuse infiltration of tumor tissue through the ciliary body and iris,  $\times 73$  Some of the spindle-shaped cells are arranged in compact manner and others are loosely bound A considerable number of macrophages which contain pigment are scattered throughout the tissue in the region of the ciliary body

The cells grown in the tissue cultures were like fibroblasts, but since we are unable to distinguish in vitro normal fibroblasts of chickens from malignant fibroblasts, and since inoculation experiments were unsuccessful, the character of these cells remains uncertain. Tissue cultures prepared from virus-induced tumors, however, invariably produced cells that were proved to be sarcoma by reinoculation into chickens.

#### PRODUCTION OF VIRUS-INDUCED TUMOR ON THE CHORIOALLANTOIS

Because the benzpyrene tumors failed to grow on the chorioallantois, it seemed essential to control this phase of the work with a virus tumor known to be transmissible. For this purpose, sarcoma 13 of Stubbs and Furth<sup>10</sup> was selected.

This virus produces sarcoma at the site of intramuscular injection with or without erythroleukosis and also stimulates endothelial cells of the blood-forming organs to neoplastic growth.

This sarcoma was readily grown on the chorioallantois in numerous subpassages. Particles of tumor that measured 1 mm in the greatest diameter were placed on egg membranes, and after ten days' incubation each was about ten times as large as it was originally. The gross and the microscopic appearance of the sarcoma on the chorioallantois are illustrated in figure 2 *A, B* and *D*. The growth was most distinct on the inner aspect of the membrane and appeared as a gray, opaque soft nodule, measuring 11 by 9 by 8 mm. On the fifth passage it was collected from the egg and inoculated into the breast of 4 chickens, in 3 of which tumors developed. In another experiment the growth was collected after the first subpassage and inoculated into 3 chickens. At the sites of inoculation in all 3 chickens tumors developed, and, of 9 chickens that were given intramuscular injections of the sarcoma grown on the egg membranes, 8 had metastases in the liver and spleen.

These experiments indicate that cells of sarcoma 13 can be readily conveyed on the chorioallantois and that the growth retains its virulence for at least five generations. Moreover, the growth on egg membranes appeared as good in the eighth generation as in the first and the fifth.

#### PRODUCTION OF TUMORS WITH CELL-FREE VIRUS OF SARCOMA 13

Attempts were made in four experiments to produce tumor on the chorioallantoic membrane by means of cell-free virus. Two of these attempts were successful (table 2).

In experiment A, the tumor extract was freed from cells by centrifugation at 3,000 revolutions per minute for fifteen minutes, and the supernatant fluid was recentrifugated at 8,000 revolutions per minute for thirty minutes in an angle

10 Stubbs, E. L., and Furth, J. J. *Exper. Med.* **61**: 593, 1935.

centrifuge The supernatant fluid was used for inoculation in chickens and for implantation on the chorioallantois The material was cell free when examined under the microscope and was sterile when cultured aerobically and anaerobically

Experiment B was carried out in a similar manner except that frozen cellular tumor material suspended in Tyrode's solution was used in the preparation of the extract The resultant supernatant fluid was concentrated by centrifugation in a Bauer and Pickels<sup>11</sup> high speed centrifuge run at 28,000 revolutions per minute for forty-five minutes

In experiments C and D, 10 Gm of tumor (sarcoma) was finely chopped and ground with sterile sand, 250 cc of Tyrode's solution was added and the suspension shaken for fifteen minutes Sand and solid particles were removed by centrifugation The supernatant fluid was passed through a Seitz E K filter, with a culture of *Bacillus prodigiosus* being used as a control This filtrate was also free from cells

In 12 of 32 egg membranes, each of which had received 0.25 cc of cell-free extract, tumors were observed (table 2) Discrete, raised gray-white opaque nodules, 1 to 3 mm in diameter, covered the inoculated areas Fragments of membrane containing the growth were reimplanted

TABLE 2—*Production of Sarcoma with Cell-Free Virus (Sarcoma 13)*

Experiment	Chorioallantois		Pectoral Muscle	
	Number Inoculated	Number with Tumors	Number Inoculated	Number with Tumors
A	8	6	3	3
B	8	0	2	2
C	8	6		
D	8	0	4	2
Total	32	12	9	7

on two successive series of egg membranes in each of the four experiments, and all produced growths of about the same character and size as those of the first passage The malignant nature of these lesions was not determined by inoculation in birds Similar lesions, however, were never produced on membranes by implantation of sterile tumor-free tissue

These experiments indicate that the virus of sarcoma 13 readily produces tumor on the chorioallantoic membrane of the chick embryo and is capable of producing a growth in the breast of the chicken

#### PROPAGATION OF SARCOMA 13 IN THE ANTERIOR CHAMBER OF THE CHICKEN'S EYE

Fragments of sarcoma 13, 1 mm in diameter, were placed in the anterior chambers of chickens' eyes under sterile precautions, and ten

11 Bauer, J H, and Pickels, E G J Exper Med 64 503, 1936

days after the transplantation an average increase of twice the initial size of the inoculum was noted in one half of the birds. A tumor that occupied practically all of the anterior chamber of a chicken's eye twenty-seven days after inoculation is shown in figure 2 C. This tumor infiltrated the iris, ciliary body and part of the choroid, and loosely growing tumor cells also invaded the posterior chamber of the eye (fig. 2 E).

Table 3 shows that 22 inoculations were made into anterior chambers of chickens' eyes, and in 11 instances progressively growing tumors developed. The percentage of successful ocular implantations was 50, as compared with 65.7 in the chicken breast and 70.8 on the chorio-allantois. These experiments show that the anterior chamber of the eye is a satisfactory site in which to grow a virus tumor. Although statistical proof is lacking, our observations indicate that the rate of growth is more rapid on the chorioallantois than in the breast of the young chicken or in the anterior chamber of the eye.

TABLE 3—*Transplantation Experiments with Sarcoma 13*

Site of Injection	Injections	Tumors	Percentage with Tumors
Anterior chamber	22	11	50
Intramuscular site	38	25	65.7
Chorioallantois	120	85	70.8

## COMMENT

Numerous birds which had been given injections of different viruses of leukosis and sarcoma were in the same animal room with chickens that were being given injections of benzpyrene. The fact that the benzpyrene-induced tumors could be passed with the greatest difficulty in chickens and not at all on the chorioallantois indicates that they do not contain any of the viruses used in other studies in this laboratory. Since we have failed to propagate the benzpyrene tumors in series, the question arises whether these growths were malignant. Microscopically they were indistinguishable from the chicken tumors that were produced by virus and from the sarcoma growths in mice. They were locally invasive, and metastases were found in 2 of the 3 chickens which received benzpyrene. Malignancy and transplantability obviously do not go hand in hand.

The success of other workers with various tumor implantations on the chorioallantoic membrane and in the anterior chamber of the eye led us to investigate whether the benzpyrene tumors could be propagated by these methods. These experiments were unsuccessful, but control experiments with the readily transplantable chicken sarcoma showed that the anterior chamber of the eye is slightly less favorable for the

propagation of sarcoma than the breast of the chicken. The chorioallantois, however, appears to be as good, if not better, than the breast as a site for the propagation of the growth.

Greene and Saxton<sup>12</sup> transplanted rabbit uterine adenoma into anterior chambers of rabbits' eyes. When tumor fragments were used, 50 per cent grew successfully. Although we worked with a virus-induced tumor of fowl, our percentage of transplants that grew is similar.

While our figures show that the anterior chamber of the eye is less favorable for the propagation of a tumor than the other two sites mentioned, the percentage of transplants showing growth is sufficiently high to demonstrate the value of this procedure. The main advantage of the method is that one may make repeated and direct observations of the tumor.

Attempts were made in a series of experiments to grow malignant lymphoid cells of mice on the chorioallantois. These experiments have so far given negative results but are still in progress.

#### SUMMARY AND CONCLUSIONS

Sarcoma was produced in 3 chickens by injection of benzpyrene. Attempts to transplant 2 of the tumors into 39 chickens were unsuccessful, and the third tumor could be transplanted in but 1 of the 47 chickens in which it was inoculated. In subpassage this tumor grew in only 1 of 10 chickens into which it was passed, and on further passage, in 1 of 13.

These tumors could not be propagated in the anterior chambers of chickens' eyes, and attempts to propagate 1 of the 3 tumors on the chorioallantoic membrane of the chick embryo were unsuccessful.

The fact that the benzpyrene-induced tumors were transplanted with such difficulty indicates that if they contained any virus they did not contain any of the readily transmissible viruses studied in this laboratory.

The virus-induced sarcoma 13 studied in control experiments was grown on the chorioallantois in 70.8 per cent, in the breast of the chickens in 65.7 per cent and in the anterior chamber of the chicken's eye in 50 per cent of the trials. The fact that the growth may be observed directly in the anterior chamber makes this a valuable site for the study of tumor propagation.

This tumor was transmitted on egg membranes for eight successive generations.

Cell-free material from sarcoma 13 produced sarcoma on the chorioallantoic membrane of the chick embryo as well as in the breast of the inoculated chicken.

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12 Greene, H. S. N., and Saxton, J. A., Jr. *J. Exper. Med.* **67**: 691, 1938.

# PRIMARY CARCINOMA OF THE LIVER

J LOESCH, M D

ONEONTA, N Y

In the majority of cases primary carcinoma of the liver is rather easily identified and classified from the distribution and characteristic gross appearance, especially when it is in the early or moderately advanced stage, or, if the nature of the lesion is more obscure, by a careful search of the other organs to eliminate a primary tumor in those structures. However, it is frequently difficult, sometimes even impossible, to classify primary carcinoma of the liver as to histogenesis.

My co-workers and I on several occasions saw so much variation of the histologic picture during routine study of such cases or even within the same case that we were prompted to subject our autopsy material to a careful scrutiny, taking several blocks from different areas of the tumor and thereby attempting to evolve certain histologic criteria which could be used for classification in atypical cases.

According to the literature, all investigators seem to have agreed on the comparatively rare occurrence of this type of neoplasm. Thus Goldzieher and von Bokay<sup>1</sup> reported 18 cases in 6,000 autopsies, Rowen and Mallory<sup>2</sup> 9 in 6,500, Clawson and Cabot<sup>3</sup> 5 in 5,100, Torland<sup>4</sup> 10 in 6,000 and Fried<sup>5</sup> 4 in 1,200.

Our material comprised 11 cases of primary carcinoma of the liver in advanced stages and 3 of the neoplasm in early stages in 3,000 autopsies. The latter (cases 2, 3 and 5) were found incidentally.

## REPORT OF CASES

CASE 1—The patient was an emaciated white man, 55 years old.

The liver was cirrhotic, its weight was 6½ pounds (2,948 Gm), contiguous to the inferior vena cava was a large necrotic tumor mass with central hemorrhage,

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1 Goldzieher, M. A., and von Bokay, Z. *Virchows Arch f path Anat* **203** 75, 1911.

2 Rowen, H. S., and Mallory, F. B. *Am J Path* **1** 677, 1925.

3 Clawson, B. J., and Cabot, V. S. *J A M A* **80** 909, 1923.

4 Torland, cited by Herxheimer, in Henke, F., and Lubarsch, O. *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1930, vol 1, p 823.

5 Fried, B. M. *Am J M Sc* **168** 241, 1924.



whitish nodules, ranging in size from that of a small pea to that of a large cherry, were distributed throughout the remaining portion of the organ

Microscopically, the tumor resolved itself into acini with delicate vascularization between these structures. The acinal cells were much smaller than the liver cells and had basophilic cytoplasm. The majority of the acini contained bile in their lumens. Single tumor acini were frequently seen in the periportal lymph spaces and in larger veins, intermingled with blood. Occasionally, some of the acinal cells showed mitotic figures with formation of giant cells. Contiguous to the tumor, the hepatic parenchyma was compressed, resulting in marked degenerative changes in the liver cells.

CASE 2—The patient was a well developed, somewhat obese white man, 62 years old.

The liver was characterized by advanced cirrhosis and showed numerous large regenerative adenomatous nodules scattered throughout, in the upper half of the midportion, between the right and the left lobe, there was an encapsulated olive-sized nodule, largely friable and necrotic.

Microscopically, the structure of the nodule was like that in case 1 except that no bile was found. Serial sections revealed capsular invasion and extension along the lymph and blood spaces, with tumor cell nests a considerable distance from the main nodule. One of the tumor foci had penetrated the wall of a small vessel and therewith gave rise to further spread of the tumor.

CASE 3—The patient was a poorly nourished white man, 60 years of age.

The liver was cirrhotic, in the center of the right lobe was a tumor node, 2 cm in diameter and encapsulated by a thick layer of connective tissue. On sections through various levels it was found that the tumor had penetrated the capsule at several points and that thrombi extended into the branches of the portal vein.

Microscopically, the cirrhotic liver presented small deposits of iron pigment in the periportal connective tissue. The structure of the tumor was like that in case 2.

CASE 4—The patient was a fairly well nourished white man, aged 62 years.

The liver was discolored diffusely greenish, it was cirrhotic, with a deep cleft along the coronary ligament, in the upper portion of the right lobe there was a large striated and lobulated tumor mass, immediately adjacent to the latter there were numerous small nodules, which compressed but did not invade the inferior vena cava or hepatic veins. Metastases were found in several thoracic vertebrae, in two situations they protruded into the spinal canal, resulting in compression of the spinal cord.

Microscopically, this tumor greatly resembled that in case 3. In addition, however, the cells were at times arranged in strings with spaces between them, suggesting hepatic sinusoids, as they were lined by slender cells resembling Kupffer cells. There were also areas where the tumor formed wide cords, with the cells varying considerably in size and shape, many of them containing acidophilic granules in the cytoplasm. Bile was nowhere demonstrable. The structure of the vertebral metastases was the same as that of the main tumor. Sometimes the variation seemed to be even greater.

CASE 5—The patient was a well nourished white man, 50 years old.

The liver was slightly enlarged, with sharp edges and a reddish brown cut surface. At the porta hepatis there was a circumscribed whitish growth, 1.75 cm

in diameter, which had broken into a large vein, filling the entire lumen with a tumor thrombus

Microscopically, the liver was that of a patient with acute leukemia. The hepatic cells contained a great deal of iron pigment, more pronouncedly at the periphery of each lobule. The tumor nodule, subdivided by several septums, had penetrated at one point the inner capsular layer and was spreading in a circular manner along the lymphatic spaces. It was immature and showed in the main a medullary arrangement, forming wide cords with fine vascularization between them. In a few areas, however, typical acini were seen. The lymph spaces were markedly distended, giving the growth an angiomatous character. Bile formation was nowhere demonstrable.

CASE 6—The patient was a well nourished white man, 62 years old.

The liver was cirrhotic, the parenchyma between the markedly thickened portal framework was replaced by whitish nodules of varying size, except for a small area in the left lobe. Most of the intrahepatic vascular spaces were invaded by tumor. Through a vein of the liver this growth extended into the inferior vena cava and was adherent to the wall of the vessel for 8 cm. After penetration of the wall at one point, it spread along the lumbar portion of the spine, invading simultaneously some of the intervertebral disks and the bodies of the second and third lumbar vertebrae. Small metastases were demonstrable in the first, second and third ribs and the clavicle on the left side, one small tumor nodule was found in the body of the sternum, and numerous ones, varying in size, were scattered throughout both lungs.

Histologically, the tumor proliferations resembled in the main those in case 2. In addition, in some situations the acini contained in their lumens granular acidophilic masses. The accumulation of these reached, especially in the central portions, such an extent that the lumens were distended and the surrounding cells flattened. Thus a picture not unlike that of a thyroid was produced. In other places the growth consisted of columns, the cells of which resembled hepatic cells. In some vessels (fig 1) the blood was frequently intermingled with tumor acini, thus accounting for the wide spread of metastases. The thrombus in the inferior vena cava and the nodules in the lungs presented the same picture as the tumor in the liver. In the bone metastases, however, a more solid arrangement predominated. The cells were pleomorphic, and their cytoplasm was distended by mucoid material, which displaced the nuclei peripherally.

CASE 7—The patient was a well nourished white man, aged 60 years.

The liver was small and cirrhotic, with ill defined whitish tumor nodules from 0.5 to 2 cm in diameter scattered throughout the organ. There were multiple metastases in the peritoneum.

Microscopically, the markedly cirrhotic liver showed small deposits of pigment in the periportal tissue. The tumor revealed great variation histologically. Thus, in one area there were mature adenomatous formations (fig 2A), many of which contained inspissated bile in their lumens and were subdivided by septums and surrounded by a connective tissue capsule. Occasionally, the latter was missing and the transition from tumor to liver columns was direct. In other areas the liver parenchyma was replaced by narrow cords of tumor cells, which stained dark blue with hematoxylin and eosin (fig 2B). The stroma was diffusely infiltrated by polymorphonuclear leukocytes. In other situations, again, broad columnar-like formations were noted, consisting of cells with the nuclei placed peripherally and

the cytoplasm a diffuse pink (fig 3 A) Finally, areas of pleomorphic elongated tumor cells were encountered (fig 3 B) Arranged in groups or single and enmeshed in young connective tissue, they simulated a sarcoma-like structure

CASE 8—The patient was a poorly nourished white man, 58 years old

The liver was cirrhotic, with whitish nodules from 0.5 to 4 cm in diameter, the largest concentration of which was in the right lobe A tumor thrombus was found in the portal vein, beginning at the fusion of the mesenteric tributaries and extending into several intrahepatic branches In the lower lobe of the right lung there was a metastasis 1 cm in diameter

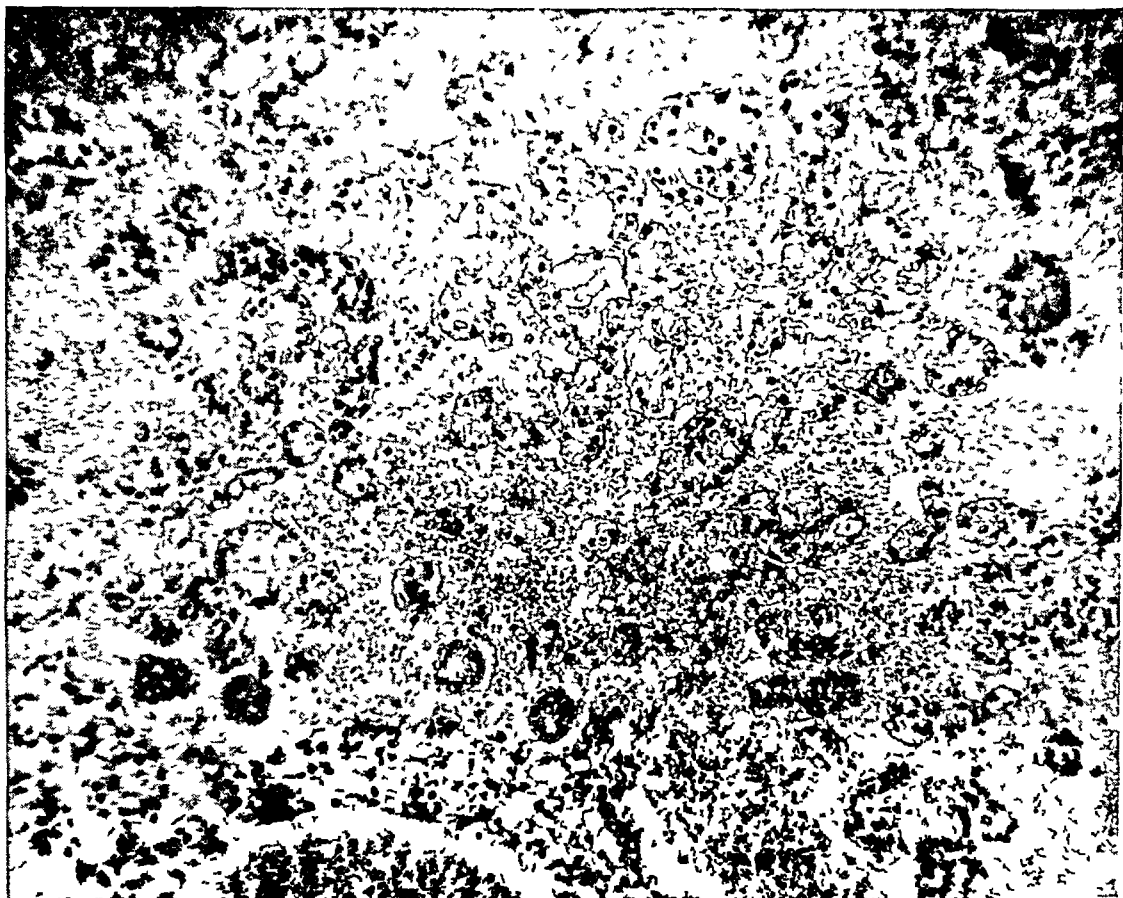


Fig 1—Tumor acini in a blood vessel

Histologically, the tumor consisted chiefly of cords of cells, varying in form and size, with the cytoplasm slightly acidophilic and the nuclei strongly basophilic Large multinucleated cells, so-called tumor giant cells, were found not only in the neighborhood of necrotic areas but also in the intact tumor Frequently they formed true tumor columns (fig 4) Some of the tumor cells contained in their cytoplasm granular debris and numerous polymorphonuclear leukocytes In other sections, again, the tumor consisted of large uninucleated cells with fat vacuoles in the cytoplasm The stroma was scant, and through it ran a fine capillary network Thus, the picture was not unlike that of hypernephroma Further, in other sections a pleomorphic cellular arrangement was seen as observed in some of the previous cases

CASE 9—The patient was a white man aged 71, fairly well nourished

The liver was markedly enlarged, with numerous nodes protruding from its surface. Nearly the entire lower portion of the right lobe was replaced by a tumor mass, 15 cm in diameter and necrotic in the center. There were several tumor proliferations in the remaining portions of the liver. A small metastasis was detected in the lower lobe of the left lung and in a lymph gland at the bifurcation of the trachea.

Microscopically, the liver revealed foci of necrosis and a central zone of congestion with atrophy of the liver cells. Peripherally there were small deposits of fat. The periportal connective tissue was but slightly increased. The tumor was arranged in cords. Some of the cellular elements resembled hyalinized liver cells, the rest were pleomorphic. The latter predominated, varied in size and had no similarity to any cell type. Some stained very dark, others were light. Along the edges of the tumor a gradual shading off into normal tissue was noted. Also, tumor giant cells and tumor invasion of vessels were frequently seen. The extrahepatic metastases revealed the picture of the primary tumor.

CASE 10—The patient was a white man aged 60 years, fairly well nourished

The liver was markedly enlarged, its weight was 6 pounds (2721.5 Gm). Numerous whitish nodes, varying in size, were scattered throughout the organ, a tumor thrombus was found in the portal vein and metastases in the portal lymph nodes.

The tumor consisted of cells that were cylindric or cuboid, in their cytoplasm they contained acidophilic granules, they were arranged either in single layers with a delicate stroma between them or in solid thick cords. In the latter, central necrosis was sometimes observed. Also, this tumor frequently showed multinucleated tumor giant cells. The metastases in the portal lymph nodes presented great similarity to the picture just described. The medullary arrangement, however, predominated.

CASE 11—The patient was a white man aged 60 years, poorly nourished

The liver was cirrhotic, with large adenomatous nodes as evidence of marked regeneration. Almost the entire right lobe was replaced by a tumor mass that was necrotic in the center. The left lobe contained a few small nodes. In the portal vein a tumor thrombus filled the entire lumen and extended into several intrahepatic branches.

The tumor was of a medullary type throughout. On longitudinal sections the cells formed broad columns, and between the latter was a delicate vascular network. Thus this growth resembled somewhat that in the previous case. Tumor giant cells were seen in almost every field. The liver cell cords adjacent to the tumor nodes were compressed, indicating expansive rather than infiltrative growth. The periportal tissue contained tumor in nearly every blood and lymph space. The tumor formations in the lungs were like those described in the liver.

CASE 12—The patient was a white woman aged 45 years, well nourished

On exploratory laparotomy the diagnosis of carcinoma of the liver was made, and a piece of tissue was removed. The histologic diagnosis was carcinoma with cells resembling liver cells. Permission for an autopsy was refused.

CASE 13—The patient was a white man of 76 years, poorly nourished

In the right lobe of the cirrhotic liver there was a large tumor mass, with numerous tumor nodules of varying size in the remaining portions of the liver. There were metastases in two portal lymph nodes.

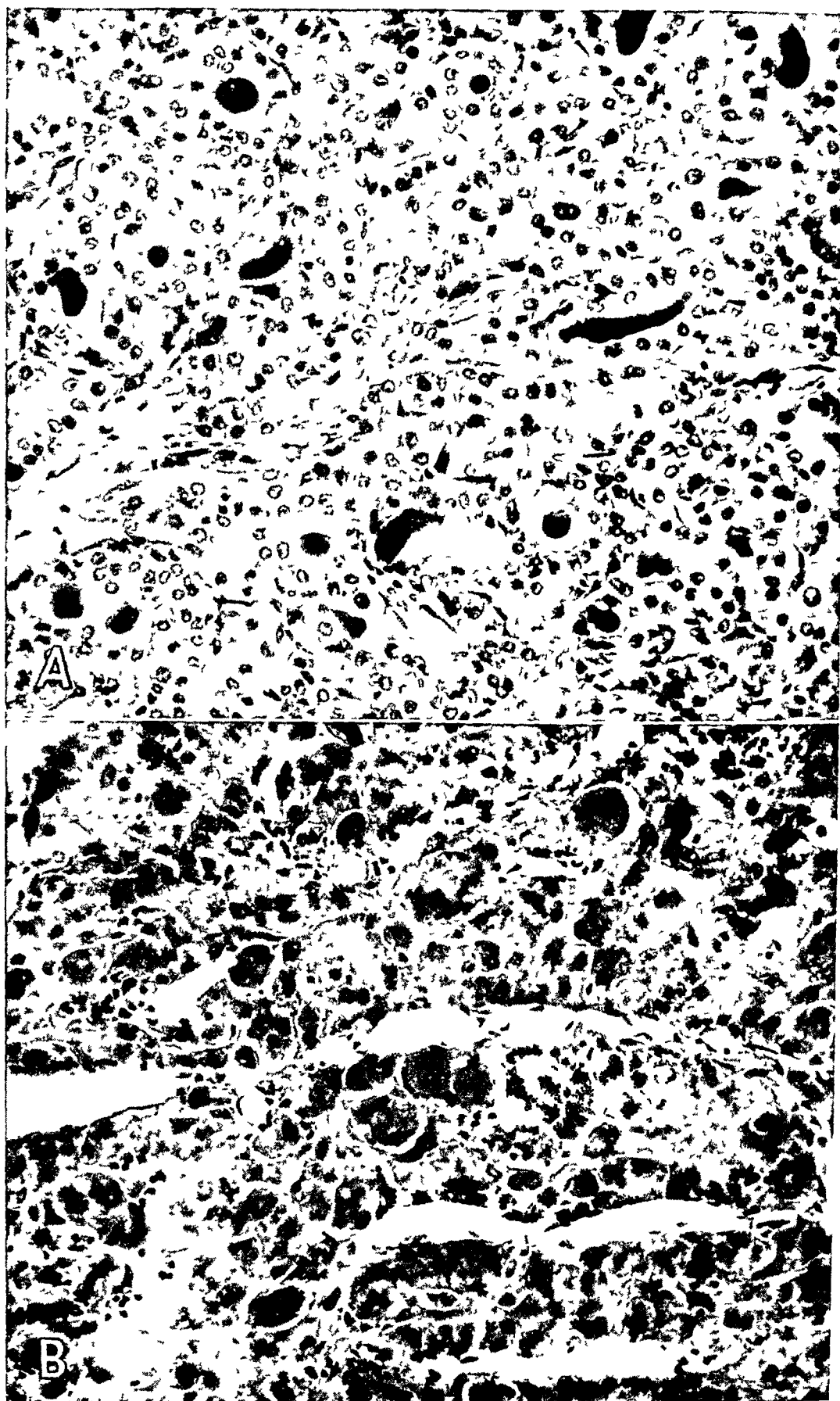


Fig 2—Variations of the histologic picture in case 7 (see text)

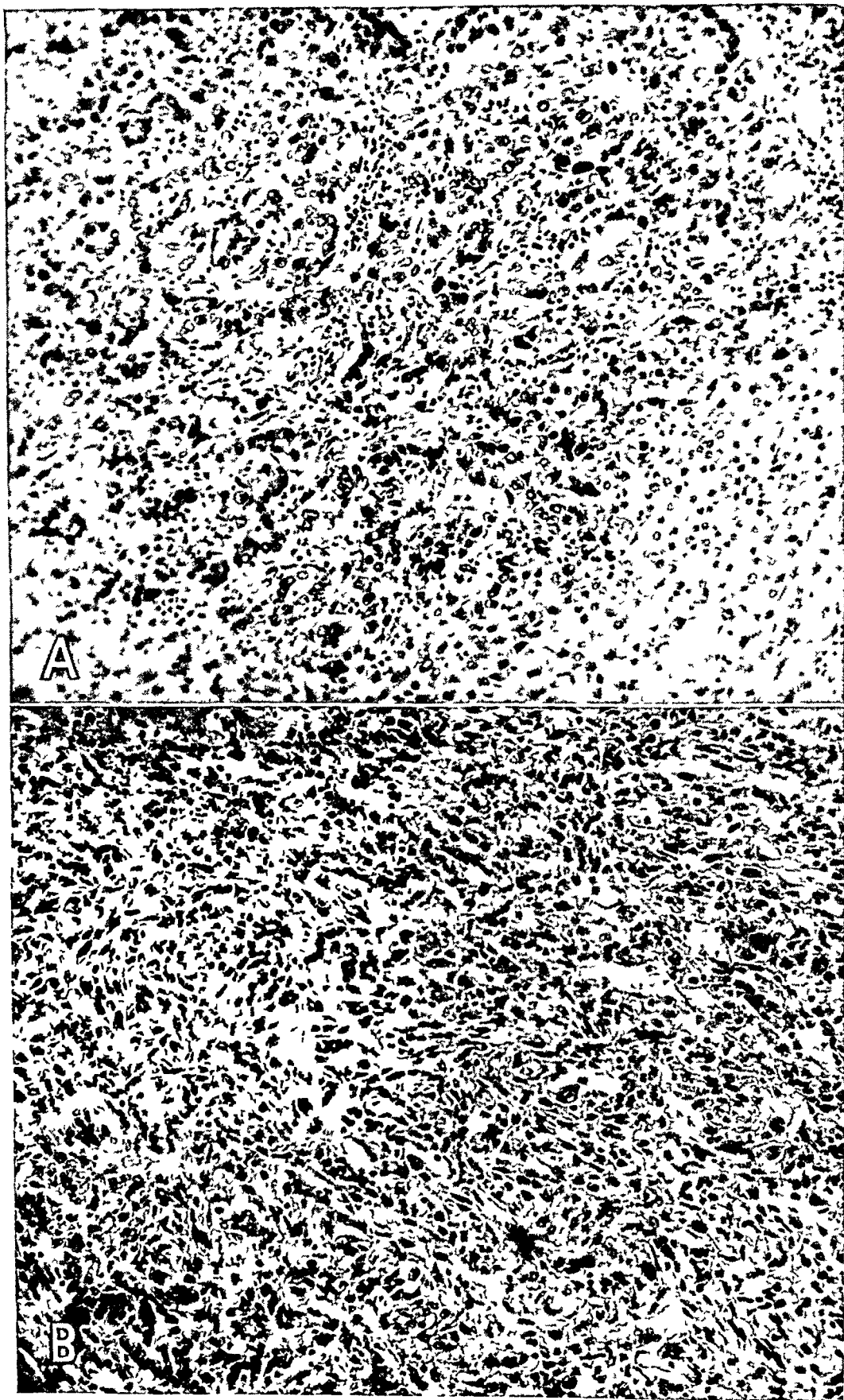


Fig 3—Further variations of the histologic picture in case 7

CASE 14—The patient was a very obese white woman, aged about 50 years

The liver was enlarged, and the surface was finely granular. On section the entire organ was studded with tumor nodes, the greatest concentration of which was in the lower portion of the right lobe.

As the last two tumors had about the same histologic picture they will be described together. The growth had a tubular arrangement consisting of one layer of cells which were at times somewhat cuboid and at other times cylindric, resembling the lining cells of bile ducts. Bile was nowhere found, also there were no tumor giant cells. Between the tubular structures, which were more

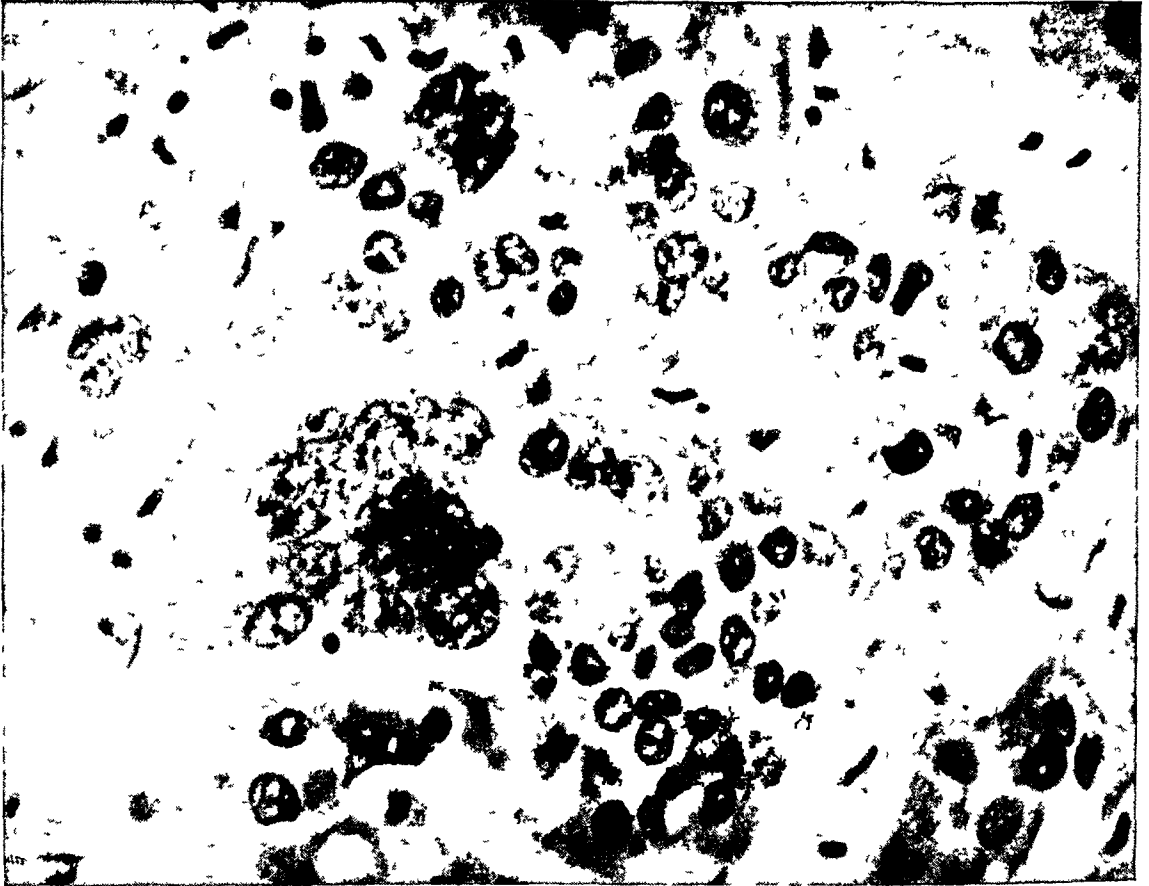


Fig 4—A tumor giant cell forming a tumor column

clearly defined in the peripheral portions of the nodes, there were broad strands of connective tissue. These were diffusely infiltrated with polymorphonuclear leukocytes and carried but scanty vascularization. The metastases in the lymph nodes had merely an imitation of the tubular structure found in the liver as the arrangement of the cells was much more dense.

#### COMMENT

On a careful analysis, giving attention to cell structure and to arrangement of cells, to the presence of stroma and to its width, to the degree of vascularization and to the functional capacity of the cells,

cases 1 to 12 were classified as cases of liver cell and cases 13 to 14 as cases of bile duct cell carcinoma. The terms "hepatoma" and "cholangioma," introduced by Yamagiwa<sup>6</sup> and used in more recent articles by Fried<sup>5</sup> and Orsos,<sup>7</sup> were omitted because they might lead to confusion inasmuch as they do not express the malignant character of the tumor and therefore might be justifiably used for the benign adenoma. If, however, such designations are used for the malignant tumors, then the attribute "carcinomatosum" or "malignum" should be added.

The liver cell type is unique. Acini formations, which characterize the more mature tumor, were found in cases 1, 2, 3, 4, 5 and 7. These contained inspissated bile in cases 1 and 7 only. Mucoid droplets in the cellular cytoplasm, resulting in collections of this material in the lumens of acini, were found in cases 4, 6, 7 and 9. In case 6 the lumens were distended in situations, the lining cells being flattened and a structure not unlike thyroid produced. This formation of mucus was not regarded as a degenerative process but as a part of the functional activity of the carcinoma cell. This belief was supported by the absence of disintegrative material in these areas. The fat deposits in cases 4 and 6 were other evidences of the functional capacity of the cancer cells. In case 5 another feature was noted, which consisted of a marked distention of the vascular network, imitating an angiomatous type of tumor. Case 7 was characterized by rosette formation with inspissated bile in the center and by columns of carcinoma cells that were distinctly basophilic. The stroma which was infiltrated by polymorphonuclear leukocytes was wide. In some areas there were columns of cells containing a mucoid material that took a violet stain with hematoxylin and eosin, and in others, cellular foci, suggesting a sarcomatous tumor. This case illustrated very clearly the fallacy of expressing any conclusive opinion as to the diagnosis based on the morphologic structure of the tumor in any one single area. My colleagues and I have been profoundly impressed by this fact in the study of this series of cases. Such terms as "sarcoma" and "carcinosarcoma" would probably have been excluded in some of the reported cases if sections from many areas had been studied. In case 8 the hypernephroma-like structure and the pleomorphism of the cells were outstanding features.

Multinucleated tumor giant cells were found in cases 1, 6, 8, 10 and 11, varying in number and size. Occasionally they formed an entire tumor column. They are, in my opinion, peculiarly characteristic of carcinoma of the liver cell type and were missed in bile duct cell carcinoma. Etiologically considered, these cells are probably in some of

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<sup>6</sup> Yamagiwa, K. *Virchows Arch f path Anat* **206** 437, 1911

<sup>7</sup> Orsos, F. *Beitr z path Anat u z allg Path* **84** 33, 1930



the cases the result of precipitated division of the nuclei without division of the cytoplasm. This seems to be the only explanation when regressive changes are not in evidence. In the majority of cases, however, I regard them, in agreement with Petersen,<sup>7a</sup> as a sign of slowly occurring cellular disintegration. This disintegration is evidenced by the granular debris in the cytoplasm and the immigration of numerous leukocytes therein. The nucleus, however, still divides, but not the cytoplasm. The stroma in nearly all these cases was delicate, carrying a fine vascular network.

The bile duct cell type showed a tubular arrangement, a uniform histologic picture, a broad stroma, no formation of bile, and tumor giant cells nowhere.

A considerable literature has accumulated dealing with the question as to whether primary carcinoma of the liver is multicentric or unicentric in origin. Adherents of the first theory were Travis,<sup>8</sup> Lohlein,<sup>9</sup> Yamagiwa,<sup>6</sup> Goldzieher and von Bokay,<sup>1</sup> Counsellor and McIndoe<sup>10</sup> and others. The chief representative of the second theory was Ribbert.<sup>11</sup> He claimed also that the spread of the tumor takes place by contiguity and along the distributing lines of the portal vein. In this statement he was supported by Heixheimer,<sup>12</sup> and Saltikow, who pointed to the frequent invasion of smaller vessels, and was followed by Wegelin,<sup>13</sup> Lissauer,<sup>14</sup> Winternitz,<sup>15</sup> Karsner<sup>16</sup> and others. Goldzieher and von Bokay strengthened the first theory by not having found in serial sections direct continuity from one growth to another. This is also true for my case 2. In the latter, as well as in cases 3, 5 and 13, in all of which the early stages of carcinoma were represented, the unicentric origin and mode of propagation were illustrated clearly, as seen from the descriptions. Theoretically, the possibility of multicentric origin has to be allowed in some cirrhotic livers, since the regenerative hyperplastic nodes are usually multiple, and the latter are closely related to the development of carcinoma. Yet it is my belief that it occurs very seldom, and but rarely will there be an occasion affording a definite proof. Thus, in case 6, which is one of diffuse liver cell car-

7a Petersen, W. Beitr. z. klin. Chir. **34** 682, 1902.

8 Travis, C. H. Bull. Johns Hopkins Hosp. **13** 108, 1902.

9 Lohlein, W. Beitr. z. path. Anat. u. z. allg. Path. **42** 531, 1907.

10 Counsellor, V. S., and McIndoe, A. H. Am. J. Path. **2** 557, 1926.

11 Ribbert, M. W. H. Das Karzinom des Menschen, sein Bau, sein Wachstum, seine Entstehung, Bonn. F. Cohen, 1911.

12 Heixheimer, in Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1930, vol. 1, p. 868.

13 Wegelin, K. Virchows Arch. f. path. Anat. **179** 95, 1905.

14 Lissauer, M. Virchows Arch. f. path. Anat. **202** 57, 1910.

15 Winternitz, M. C. Johns Hopkins Hosp. Rep. **17** 143, 1916.

16 Karsner, H. T. Arch. Int. Med. **8** 238, 1911.

cinoma, a multicentric origin could readily be considered, as many tumor nodules were of the same size. But this probably was due to the resistance of the thick portal framework, from which they could be pulled out with ease. The diffuse involvement, on the other hand, can be explained without any hesitation on the basis of the great number of carcinomatous acini found intermingled with the blood of the portal vein.

Intrahepatic metastatic dissemination occurred through the small blood vessels, lymph spaces and lymph vessels. As the latter were invaded along the large vessels, a spread, accompanying the portal ram-

*Vascular Invasion and Extrahepatic Metastases of Primary  
Carcinoma of the Liver*

Case	Branches of Hepatic Vein	Inferior Vena Cava	Branches of Portal Vein	Portal Lymph Nodes	Perito- neum	Skeleton	Lungs	Bronchial Lymph Nodes
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	+	0	0	0	0	0
4	0	0	0	0	0	Thoracic part of spine	0	0
5	0	0	0	0	0	0	0	0
6	+	+	+	0	0	Left 1st, 2d, 3d rib and clavicle body of sternum 2d and 3d lumbar vertebrae	Bilateral	0
7	0	0	0	0	+	0	0	0
8	0	0	+	0	0	0	Lower lobe of right lung	0
9	0	0	0	+	0	0	Lower lobe of left lung	+
10	0	0	+	+	0	0	0	0
11	0	0	+	0	0	0	Bilateral	0
12	0	0	0	0	0	0	0	0
13	0	0	+	+	0	0	0	0
14	0	0	0	0	0	0	0	0

fications, resulted. Tumor thrombi growing into the branches of the portal vein against the blood stream and extending even into the main trunk were another source of propagation. They were observed in 6 of these cases.

One instance was noted in which a tumor thrombus extended from a hepatic vein into the inferior vena cava and penetrated the wall of the latter at one point.

In order to get a clear view of the extrahepatic metastatic distribution the table may be referred to. It seems strange that metastases could be demonstrated in the regional lymph nodes in 3 cases only. The peritoneum was involved once, the lungs four times, a bronchial lymph node once and the skeleton twice, in a case in which the skeleton was

involved epidural extension from the thoracic portion of the spine resulted in compression of the spinal cord

Many of the investigators of the subject called attention to the fact that cirrhosis was present in a majority of cases (Eggel, cited by Heixheimer<sup>12</sup>, Rowen and Malloy<sup>13</sup>) or in all of them (Strong and Pitts<sup>17</sup>, Kaisner<sup>16</sup>, Wintermütz<sup>15</sup>) It was found in 12 of the 14 cases reported here Wegelin<sup>13</sup> and Lohlein<sup>9</sup> believed the cirrhosis to be secondary to the carcinoma, as a reaction to irritation due to the presence of the tumor At present, however, all investigators agree that cirrhosis is the primary condition and a predisposing factor One must, however, recognize that slight proliferation of connective tissue takes place around the tumor, as was pointed out by Counsellor and McIndoe,<sup>10</sup> Wintermütz<sup>15</sup> and others But this never leads to a picture of diffuse cirrhosis An example in the present series was seen in case 5, in which a distinct connective tissue reaction was noted in the immediate vicinity of the tumor but was not demonstrable in the uninvolved parts of the liver

It is a well established fact that destruction of parenchyma is followed by regeneration to a varying degree In the more pronounced lesions the latter seems to follow with greater ease Then loss of control of growth, at one time or another, results in the formation of a tumor

Comparing the 12 cases reported here with 94 cases of atrophic cirrhosis, covering the same period, I arrived at the conclusion that on the average primary carcinoma of the liver developed in 1 of every 8 cases of the latter number Pigmentary cirrhosis, which Mallory found frequently associated with carcinoma of the liver, was of no etiologic significance in the present series, but slight pigment deposits were occasionally seen in the periportal connective tissue, as in cases 4 and 7 Those in the liver cells in case 5 were unquestionably due to destruction of red cells during the course of the acute leukemia Since adenomatous hyperplasia was absent in this case, the tumor originated presumably in an embryonic rest or in a clump of liver cells which were separated from the surrounding parenchyma by connective tissue, the regulatory control being lost at a later period The truth in either case cannot be determined with any degree of certainty This case, then, may be grouped with those reported by Hedinger, Helvestine, Wiest and others, since primary carcinoma of the liver without cirrhosis is generally found in young persons

#### SUMMARY

Fourteen cases of primary carcinoma of the liver are reported, 12 of the liver cell and 2 of the cholangiocellular type The hepatocellular type is unique in the variation of the microscopic picture, not only in

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17 Strong, G F, and Pitts, H H Arch Int Med 46 105, 1930

different cases but also frequently in the same case, the cholangiocellular type is of a uniform structure, with an adenocarcinomatous picture

Metastases within the liver were demonstrated in the early stage of the development of the primary tumor. The intrahepatic spread of the growth took place along the portal vessels, lymph spaces and smaller blood vessels and from tumor thrombi of the proximal branches and the main trunk of the portal vein. In case 6 a tumor thrombus extended along a hepatic vein far into the vena cava inferior, perforating the wall of the latter at one point.

Extrahepatic metastases were not uncommon, their distribution is seen in the table.

Carcinoma of the liver is unicentric in origin, as was clearly demonstrated in the study of the early cases. The same explanation is also compatible with diffuse involvement, as seen from the descriptions given in foregoing sections.

In 3,000 autopsies 94 cases of atrophic cirrhosis were found, in 12 of which primary carcinoma was demonstrated, 2 additional cases of primary carcinoma of the liver without cirrhosis were demonstrated, the apparent origin being an embryonic rest or a clump of cells separated from the mother tissue.

It was found twelve times in males and twice in females. The average age of the patients was 59.5 years.

# CLASSIFICATION AND PATHOLOGY OF RENAL DISEASE IN THE DOG

COMPARISON WITH NEPHRITIS IN MAN

FRANK BLOOM, DVM

FLUSHING, L I, N Y

The problem of Bright's disease in man has been the subject of intensive experimentation and research. Numerous investigators have utilized the dog in the artificial production of nephropathies. On the whole, it is true that the renal lesions induced have little resemblance either to the glomerulonephritis which occurs in man or to the renal inflammatory disease which the dog suffers naturally. It is desirable to be familiar with this spontaneous renal disease of the dog in order to interpret better any experimentally produced nephropathy. Furthermore, renal disease occurs in this animal with comparatively great frequency, and if this fact is not recognized, incorrect conclusions may be drawn from the results of any experimental investigations in which the kidneys are examined.

The principal renal inflammatory disease of the dog is true interstitial nephritis, which may be acute, subacute or chronic. When acute, it closely resembles in many respects the acute interstitial nephritis in man which occasionally occurs as a complication of scarlet fever and other acute infections. The so-called chronic interstitial nephritis in man must not be confused with the true chronic interstitial nephritis of the dog. In the latter, the process has its inception in the interstitial tissue, and the tubular and glomerular changes are purely secondary. Aschoff's<sup>1</sup> statement that in some cases contracted kidney in man may commence from acute interstitial nephritis, while not generally believed, is further controverted by the fact that true chronic interstitial nephritis in the dog is easily differentiated from contracted kidney of man resulting from glomerulonephritis or arterial disease. It is thus an interesting fact of comparative pathology that interstitial nephritis which is of very minor importance in man should be of such great significance in the dog, while glomerulonephritis is of no importance in this animal but is the dominant renal inflammatory disease of man.

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<sup>1</sup> Aschoff, K. A. L. *Pathologische Anatomie*, ed 6, Jena, Gustav Fischer, 1923.

In a perusal of the medical literature it is noted that the fact that the dog suffers principally from interstitial nephritis is generally not recognized and that many descriptions are given of the spontaneous renal disease of this animal which do not closely correspond to that which actually occurs. Veterinary textbooks and writers with few exceptions also give an erroneous concept of nephritis in the dog. The terms used and the descriptions seem to have been borrowed from the older medical textbooks.

#### LITERATURE

The older veterinary pathologists were fully aware that the dogs frequently showed renal lesions at postmortem examination. In most cases the description of the lesions was based purely on macroscopic examination, and it remained for Davis<sup>2</sup> to be first to describe interstitial nephritis in the dog. Joest<sup>3</sup> and Nieberle and Cohrs<sup>4</sup> stressed the frequent occurrence of interstitial nephritis in dogs. Henschen<sup>5</sup> gave a good survey of the older literature. Muller,<sup>6</sup> Brumley,<sup>7</sup> Hutyra, Marek and Manninger<sup>8</sup> and Jakob,<sup>9</sup> in their descriptions of canine nephritis, placed exceedingly little stress on interstitial nephritis.

Workers on experimental nephritis also have stressed the great frequency of spontaneous renal disease in dogs, though in most instances either the author did not state what type was present or the pathologic description is faulty. Microscopically Dayton<sup>10</sup> found 1 of 21 dogs with normal kidneys. However, he described the histologic changes as glomerulonephritis and, in the more chronic cases, sclerosis of the arteries. Interstitial changes, such as fibrosis and round cell infiltrations, were also mentioned but no recognition was given that these were primary. MacNider<sup>11</sup> in a series of 237 dogs found 42

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2 Davis, U. Die histologischen Veränderungen bei der Nephritis des Hundes, Dissert., Berne, E. Hess, 1908.

3 Joest, E. Spezielle pathologische Anatomie der Haustiere, Berlin, R. Schoetz, 1924, vol. 3.

4 Nieberle, K., and Cohrs, P. Lehrbuch der speziellen pathologische Anatomie der Haustiere, Jena, Gustav Fischer, 1931.

5 Henschen, F., in Joest<sup>3</sup>.

6 Muller, G. A. Diseases of the Dog and Their Treatment, ed. 5, translated by A. Glass, Chicago, A. Eger, 1926.

7 Brumley, O. Diseases of the Small Domestic Animals, Philadelphia, Lea & Febiger, 1938.

8 Hutyra, F., Marek, J., and Manninger, R. Special Pathology and Therapeutics of the Diseases of Domestic Animals, translated by C. F. Marshall and C. M. Ottley and edited by J. R. Greig, J. R. Mohler and A. Eichhorn, London, Bailliere, Tindall & Cox, 1938.

9 Jakob, H. Innere Krankheiten des Hundes, Stuttgart, Ferdinand Enke 1924.

10 Dayton, H. J. M. Research **31** 177, 1914.

11 MacNider, W. deB. J. M. Research **34** 177, 1916.

nephropathic. He described the renal lesions as primary glomerulonephritis with absence of acute inflammatory glomerular changes. He made no mention of interstitial round cell infiltrations. To term such lesions glomerulonephritis is certainly contradictory to the type of renal lesions the dog commonly suffers from. Winternitz and Quinby<sup>12</sup> described spontaneous nephritis in a dog which closely resembled interstitial nephritis. However, they stated further that while spontaneous renal lesions are not uncommon in dogs occasionally extensive anatomic changes occur that are similar, even in their detail, to those found in the progressive nonsuppurative types of nephritis in man. Hartman, Bolliger and Doub<sup>13</sup> in a study of roentgen ray nephritis in dogs stated that one of the reasons that dogs were selected for their experiments is that the animals are less notorious for incidence of spontaneous nephritis. The evidence is to the contrary, renal lesions being more frequently found than is commonly believed.

Since this paper was written, Whipple and Robscheit-Robbins<sup>13a</sup> have stated that 11 per cent of the dogs in their anemia colony had glomerulonephritis at autopsy. Undoubtedly these animals had interstitial nephritis, and it would indeed be remarkable if glomerulonephritis were found in so many dogs.

#### MATERIAL AND METHODS

This report is based on observations on a series of 4,123 dogs of all ages and breeds that were brought to the hospital for examination and treatment. Of this number, 274 suffered from clinical renal disease, a diagnosis confirmed by laboratory examinations of the blood and urine, and were brought to the hospital expressly because of the uremic symptoms. Of these, 70 died or were killed because the condition was deemed incurable. In another group of 200 animals that died of various causes and in which uremic symptoms were absent, histologic examinations of the kidneys showed lesions of focal interstitial nephritis in 108 dogs.

Necropsies were made immediately and the tissues fixed in a 10 per cent dilution of neutral solution of formaldehyde U. S. P., Zenker's fluid, Maximow's fluid and Flemming's solution and were stained with hematoxylin and eosin, the Lee-Brown modification of Mallory's aniline blue,<sup>13b</sup> Wright's stain, Gram's stain, iron-hematoxylin, Van Gieson's stain, Weigert's stain and Wilder's silver stain for reticulum.<sup>13b</sup>

#### GENERAL OBSERVATIONS

The clinical features of the disease and the laboratory studies of the blood and urine have been described elsewhere.<sup>14</sup> The symptoms

12 Winternitz, M. C., and Quinby, W. C. *J. Urol.* **1** 139, 1917.

13 Hartman, F. W., Bolliger, A., and Doub, H. P. *J. A. M. A.* **88** 139, 1927.

13a Whipple, G. H., and Robscheit-Robbins, F. S. *J. Exper. Med.* **69** 485, 1939.

13b Lee, B. *The Microtome's Vade-Mecum*, Philadelphia, P. Blakiston's Son & Co., 1937.

14 Bloom, F. *J. Am. Vet. M. A.* **44** 679, 1937, *Cornell Vet.* **27** 130, 1937, *Lederle Vet. Bull.*, March-April and May-June, 1938, vol. 7.

in general are those of uremia. Edema and hematuria are exceedingly rare. Cardiac hypertrophy is usually absent, and ophthalmoscopic examination of the fundi reveals no vascular changes. Convulsions do not occur, though muscular twitchings are sometimes present. The etiologic factors are unknown, though a large variety of causes have been implicated. The disease occurs more frequently in male animals, and practically all dogs over 8 years of age evidence some degree of interstitial nephritis.

#### CLASSIFICATION

The following classification is proposed, based on the histologic examinations.

##### I Noninflammatory nephropathies

###### 1 Nephrosis

(a) Mild, moderate and severe (necrotizing)

(b) Amyloid

##### II Inflammatory nephropathies

###### 1 Suppurative nephritis

###### 2 Interstitial nephritis

(a) Acute, subacute and chronic

Glomerulonephritis and arteriosclerotic kidneys have not been encountered in this group of animals. One kidney showed spotty calcification of the larger arteries, involving principally the media and closely resembling Monckeberg's sclerosis. Lipoid nephrosis is unknown in the dog. In the group of 70 animals which died of uremia, histologic examination of the kidneys showed the following percental incidence of the different nephropathies: necrotizing nephrosis, 28, amyloid nephrosis, 14, suppurative nephritis, 71, and interstitial nephritis, 88.7.

#### PATHOLOGY

Nephrosis and suppurative nephritis are similar in their histologic aspects to the same conditions in man and therefore do not require special description.

The following discussion therefore is a discussion of interstitial nephritis. Macroscopically, the kidneys show a variety of appearances depending on the type of disease present. The acute focal type is characterized by the presence in the cortex and, if the condition is of the severer type, in the outer zone of the medulla of small pinpoint to pinhead sized areas of a whitish gray color on the surface. The acute diffuse type shows these areas increased in size and in number. If the nephritis is of a mild and focal type, the macroscopic appearance may be entirely normal. The capsule peels easily, and there is rarely any



bulging on section. The cortical markings are usually distinct but they may be obscured if the infiltrations are dense and extensive. The color of the kidney is usually normal, though occasionally it is a mottled deeper red. In healed focal nephritis, the kidney appears normal with the exception of occasional pale gray scars. The number of scars depends on the original severity of the disease.

The subacute form offers a variety of appearances and in some cases is difficult to distinguish from the chronic form. Generally, the number of scars is increased, and this produces an irregular nodular surface. The capsule is usually adherent to the scarred areas, and the organ is firmer on palpation. Irregularly scattered, particularly in the cortex, are small grayish areas. The striations are obscured in the areas of infiltration and scarring.

In the chronic form, the kidney is shrunken and contracted. It is fibrous and firm and cuts with difficulty. The capsule is commonly thickened and adherent, though this is not an absolute rule, it may strip easily. The kidney is practically always a uniform pale tan color. The surface is nodular, though the size of the nodules varies considerably in different kidneys. The cortex is irregularly decreased in size, and the markings are usually obscured and obliterated. Frequently there are present smaller and larger cystlike structures in the medulla and occasionally in the cortex.

Microscopically, the picture is also variable but in general consists of the true inflammatory changes of infiltration and proliferation. The infiltration is principally that of the interstitial tissue by mononuclear cells, and the proliferation is chiefly that of fibroblastic formation of connective tissue. The infiltrating cells have the appearance of lymphocytes, though some closely resemble plasma cells. The nucleus is oval and may be situated in the central or in the peripheral portion of the cell. In fresh infiltrations these cells are large, the nuclei contain less chromatin and therefore stain lighter. In old infiltrations the cells are smaller, the chromatin material of the nuclei is more compact, and the nuclei stain very deeply and appear pyknotic. The cytoplasm contains no granules, and on vital staining with trypan blue these cells do not take up the stain. In some cases neutrophils are present in the infiltrations, though the predominant cells are the lymphocytes. The infiltrations may be perivascular, periglomerular and intertubular in distribution.

The number of infiltrative cells depends on the acuteness and extent of the nephritis. In the focal type the infiltrations vary from several to large numbers of lymphocytes scattered irregularly throughout the kidney. Most of the cellular collections are located in the cortex and the outer stripe of the outer zone of the medulla. In the acute diffuse type the infiltrations are particularly dense and heavy. The tubules in the

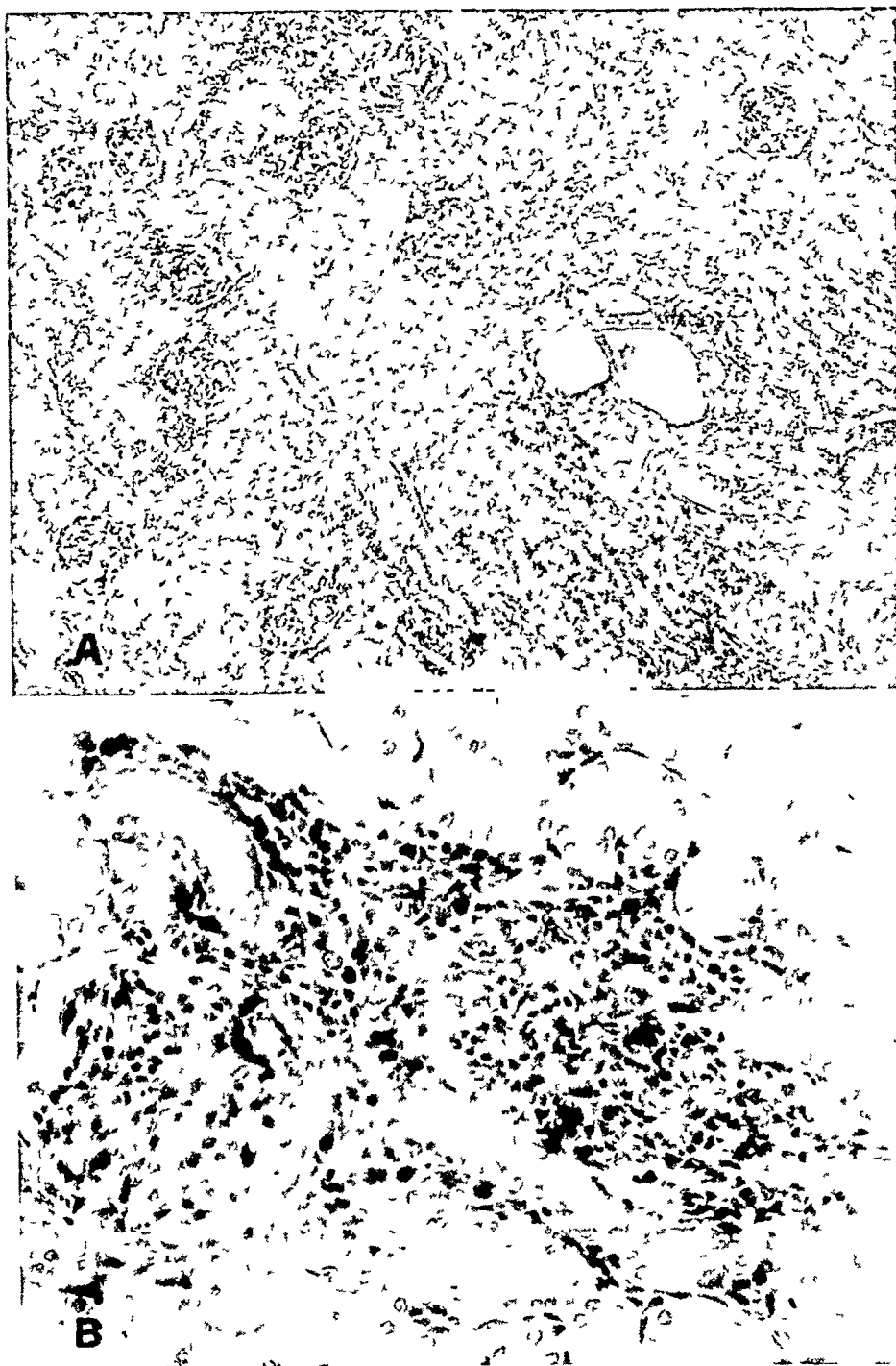


Fig 1—*A*, acute focal interstitial nephritis, hematoxylin and eosin,  $\times 60$ . Note scattered collections of lymphocytic cells in focal areas. The glomeruli and tubules are essentially normal. *B*, greater magnification of the lymphocytic infiltration of *A*, Wright's stain,  $\times 280$ .

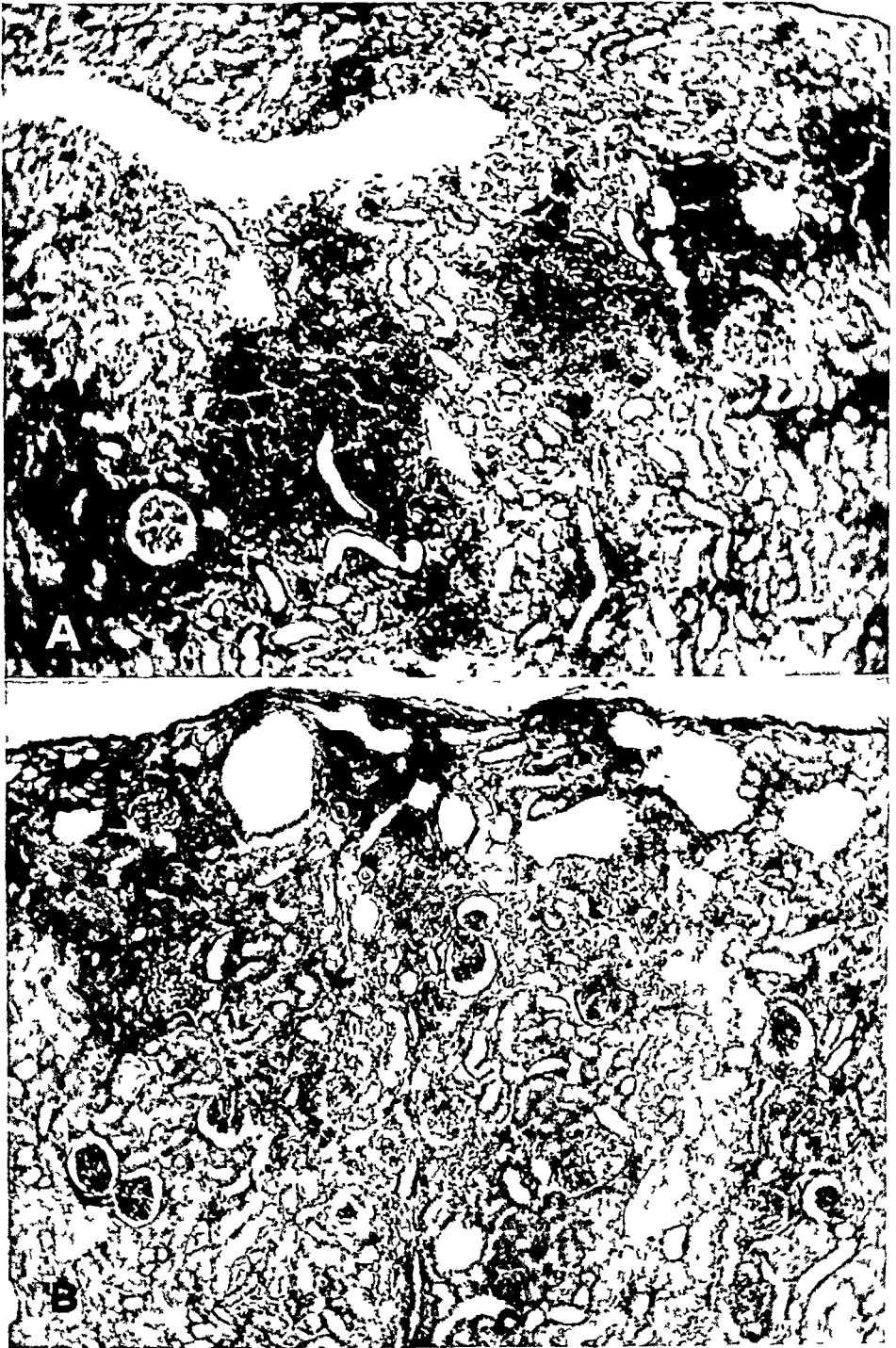


Fig 2—*A*, acute diffuse interstitial nephritis, hematoxylin and eosin,  $\times 60$ . Note very heavy collections of lymphocytic cells diffusely distributed throughout the organ. The glomeruli even though included in the areas of lymphocytic infiltration are normal. The tubules in the heavily infiltrated areas have disappeared. *B*, focal scar extending from the outermost portion of the cortex to the medulla, hematoxylin and eosin,  $\times 60$ . Several of the included glomeruli are fibrosed. Outside the scar the renal parenchyma appears normal.

infiltrated areas may be displaced or destroyed, usually the epithelial nuclei persist in moderate numbers. The reticulum in these areas is usually sparse. In general the infiltrations are lighter in the inner zone of the medulla and rarely occur as large groups of cells.

The glomeruli and blood vessels show practically no changes with the possible exception of mild congestion in some cases, even though heavily surrounded by inflammatory cells. The tubules involved evidence degenerative changes of different degrees, though many may be normal. In the focal type the tubular changes are of a mild nature, consisting of cloudy swelling and slight vacuolation of the cytoplasm. In the diffuse type the parenchymatous degeneration may be severe, with necrosis of the cells and disappearance of their nuclei. The degenerative changes are most prominent in the proximal convoluted tubules. Hyaline casts are commonly found and occasionally are of a granular nature with nuclear remnants. In the cortex, particularly, are frequently present regenerated epithelial cells, which contain large oval nuclei having a vesicular structure and boundaries that are indistinct or absent. Many times mitotic figures are present.

The aforementioned changes can terminate in several ways. 1. They may disappear completely by resolution and absorption. 2. They may continually progress until more of the renal structure is involved. 3. They may heal with the production of connective tissue.

The formation of scars is frequently met with in routine histologic examination of dogs' kidneys. They arise in the following manner. Fibroblasts appear in the areas of lymphocytic infiltration, with the formation of fibrous connective tissue. As the fibrosis increases, there is a corresponding reduction in the number of round cells, though they rarely disappear entirely. With the Van Gieson and the Weigert stain, the connective tissue is of the fibrous type and is richly cellular in the earlier stages of the process. The tubules and glomeruli in the scarred areas are also altered, the tubules may disappear or persist as scars or they may become dilated, showing a thin atrophic epithelium, and frequently they contain hyaline casts, the glomeruli may be distorted, fibrosed or hyalinized and may show well advanced periglomerular fibrosis.

In some cases fresh lymphocytic infiltrations appear, and the process of infiltration and scarring appears to be continuous. This constitutes the subacute form. In such kidneys the pathologic alterations are decidedly more advanced than in the acute type. The fibrosis is increased, and there are larger numbers of affected glomeruli and distorted and dilated tubules. Throughout the kidney are fresh lymphocytic infiltrations with the corresponding tubular changes. However, a large amount of the renal tissue is normal and free from alterations.



Fig 3—*A*, chronic interstitial nephritis, hematoxylin and eosin,  $\times 60$  The cortex shows diffuse proliferation of interstitial connective tissue with scattered lymphocytic infiltrations. The tubules show varying distortions, from collapse to dilatation. The epithelium of the dilated tubules has been reduced to a thin, atrophic layer. Some of the glomeruli are fibrosed, while others are distorted. *B*, chronic interstitial nephritis, hematoxylin and eosin,  $\times 60$  Section of medulla showing the distended collecting tubules with hyperplastic epithelium. There is diffuse proliferation of intertubular connective tissue.

Eventually the subacute form may result in chronic interstitial nephritis. In the latter, the quantitative structural changes vary considerably. The connective tissue proliferation is extensive in all cases, though in some the cortex is more affected, and in others the medulla is more involved. Large areas of the organ may be converted into masses of fibrous collagen. Scattered throughout the fibrosis are collections of lymphocytic cells with no definite distribution. The glomerular changes are similar to those found in the focal scars but are more extensive. However, in many kidneys the majority of the glomeruli show little change. The tubular changes vary considerably. In clinically compensated conditions the cortex may appear almost normal, though the medulla is extensively affected. The number of dilated tubules is greatly increased. This dilatation is exceedingly irregular, and the epithelium is very flattened. The convoluted tubules that persist in their apparent normal structural basis show decided parenchymatous degeneration, and their lumens contain granular material. Fairly constant in the inner zone of the medulla is the presence of dilated hyperplastic collecting tubules that have a glandlike appearance. The cell boundaries of such tubules are indistinct, and the nuclei are arranged in irregular rows. In the cortex and outer stripe of the outer zone of the medulla are occasionally present hypertrophied tubules with tall columnar epithelium. In some cases calcareous deposits are present in the fibrotic pericapsular tissue and around the atrophied tubules. In all cases the arteries are normal and show no intimal changes even though surrounded by inflammatory tissue.

#### SUMMARY

In contrast to what is observed in man, in the dog, interstitial nephritis is the most common renal inflammatory disease and may be acute, subacute and chronic. The initial pathologic process consists of lymphocytic infiltrations in the interstitial tissue and is perivascular, periglomerular and intertubular in distribution. Such infiltrations may be replaced partially or completely by fibrous connective tissue. In some cases fresh lymphocytic infiltrations appear, with further production of fibrosis, so that eventually the entire kidney may be involved. In such kidneys there are extensive fibrosis, secondary glomerular change and dilatation of tubules, showing thin, atrophic epithelium. In addition, some tubules may be hypertrophied and others hyperplastic.

Glomerulonephritis and arteriosclerotic kidneys practically never occur in the dog.

# Case Reports

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## CERVICAL POLYPS IN BOTH OF PRESUMABLY IDENTICAL TWINS

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Tumors occurring in twins are of great interest, both when the twins are of the one ovum and when they are of the two ovum type, and also when one twin only and when both twins are affected. All such cases should be reported. For that reason we are putting on record a case of cervical polyps in twins who we think are probably monozygous because of the following points of resemblance: (1) They were indistinguishable before puberty. Later, one twin gained more in weight than the other, thus altering their similarity to some degree. (2) Both now have hazel eyes with (3) marked arcus senilis, (4) the hair in both shows the same amount of graying, (5) the skin in both has the same sallow tint, (6) both have arthritis of the knees, (7) both have arteriosclerosis, (8) both react similarly to the test of taste for phenylthiocarbamide, both agreeing that it is practically tasteless, only "a little bitter."

Mrs. C. B., married and with children (her husband was a drunkard), was first seen at the age of 59. She had had a prolapsed uterus for thirteen years. Her menopause began at the age of 49. She had slight bleeding for six months before she came for examination, which revealed perineal lacerations and a cervical polyp. The lacerations were repaired, and the polyp was removed. She had been well, without any return of the polypous growth, for seven years.

The twin sister of Mrs. C. B., Mrs. L. S., married and with children (her husband also was a drunkard), was examined at the age of 64, at which time she weighed 65 pounds (29.5 Kg.) more than her sister. She had had slight irregular bleeding for four years and feared she had cancer. Examination showed a moderate cystocele and a rectocele, and also a cervical polyp, which was removed. It was considerably larger than her sister's had been, which was to be expected inasmuch as the sister's had been operated on six months after the onset of symptoms, and hers, not until four years after the onset.

Four years later a second polyp developed, which was removed. For the pathologist's report which follows, we are indebted to Dr. Helen Ingleby, of the Woman's Medical College of Pennsylvania: "The polyp contains dilated glands embedded in cellular connective tissue. In some places the stroma is packed with inflammatory cells and the epithelium is undergoing metaplasia toward the squamous type. The cells are large and swollen, and, although they have multiplied, they appear to be degenerating and not neoplastic. There is no evidence of malignant change. The diagnosis is cervical polyp showing severe inflammation."

The patient also has a lump developing in her breast, for which she has been treated by roentgen rays, her physician preferring this type of therapy because of her very high blood pressure. Hence no pathologic diagnosis of the variety of this tumor of the breast is available, there having been no biopsy. The first twin, Mrs. C. B., has as yet no signs of any lump in her breast.

Cervical polyp and prolapse of the uterus are apparently not rare conditions. Dr Catharine MacFarlane, of Philadelphia, has given us the following data. On a gynecologic service there of 400 women a year, 2.5 per cent, or 10, a year exhibit prolapse of the uterus. Cervical polyp is encountered in about the same number, namely, 2.5 per cent. The true incidence of the latter is higher, however, being observed in about 6 per cent of gynecologic patients, as some patients are treated in physicians' examining rooms, without admission to a hospital. Inasmuch as not all women are gynecologic patients, these figures do not give the incidence of these conditions in women in general. The presence of two such conditions as cervical polyp and lax vaginal walls in two sisters with presumably the same inheritance would be expected if each condition had a definite genetic basis but would be found relatively seldom in two sisters if the two conditions were dependent wholly on extraneous factors. Both probably have a genetic basis, modifiable perhaps to greater or lesser degree by extrinsic causes.

There has been no other report in the literature, as far as we have been able to ascertain, of polypoid growth on the cervix in twins. One case of prolapse of the uterus, grade 3, and cystocele, grade 2, in both of identical twin sisters has been reported by Hines and Piper<sup>1</sup> of the Mayo Clinic.

#### SUMMARY

Cervical polyps, benign in character, were found in twin sisters who were presumably of the one ovum type. They occurred at approximately the same age in both patients. Both had lax vaginal walls, resulting in prolapsed uterus in one twin and in cystocele and rectocele in the other. In one twin a tumor has developed in the breast, there is as yet no sign of tumor of the breast in the second twin.

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<sup>1</sup> Hines, E. A., Jr., and Piper, M. C. Proc. Staff Meet., Mayo Clin. **12**: 815, 1937.



## FETAL PERICARDITIS

R W KELLEY, M D, ST LOUIS

That fetal pericarditis is rare is shown by a review of the rather extensive literature regarding fetal infections. Instances of fetal endocarditis are common, and the possible association with the production of congenital heart disease has been frequently discussed. Only 1 case of fetal pericarditis has been found recorded in the literature. This was reported by Job, Lévy and Morlot<sup>1</sup>

In this instance the infant was born at term, after a normal pregnancy. The labor was normal. Only after great difficulty were respirations initiated, and the infant remained cyanotic till death, which occurred four hours after delivery. At autopsy the pericardial sac contained a serofibrinous exudate and the epicardial surface of the heart showed small opaque plaques. The subpericardial tissues were edematous. In addition, there was acute fibrinous endocarditis, with verrucous vegetations on the mitral and tricuspid valves. The Wassermann reaction of the mother's blood was negative. There is no report of cultures taken or of microscopic studies. This infant had large areas of desquamation on the palms and soles, which led the authors to suspect syphilis as the etiologic agent.

The case to be reported here is the first instance of fetal pericarditis in approximately 7,000 autopsies performed by this department.

### REPORT OF A CASE

A 33 year old colored woman, married, was first seen in October 1924. At that time the Wassermann reaction was positive and she received one course of treatment with a preparation of bismuth and with arsenicals. In August 1935 she was given a "rest," after which she did not return for further antisyphilitic treatment. All Wassermann and Kahn tests subsequent to the first one, including those made on May 27, 1935, were negative. On Sept 17, 1935, she sought treatment for a profuse vaginal discharge, the result of chronic endocervicitis, which had been present for two or three years. She was treated by potassium permanganate douches and given pyridium by mouth. On May 20, 1936, a diagnosis of pregnancy was made, the expected date of confinement being December 20. On May 27 a gonococcic complement fixation test was returned as being 4 plus. No cultures of the cervical discharge were made. In July the patient complained of profuse vaginal discharge and of considerable pain on urination. On August 30 at the St. Louis Maternity Hospital she was delivered, by full breech presentation, of a stillborn girl. The puerperium was reported as being uneventful. The placenta was reported as premature. No mention of placentitis was made.

The father of the stillborn infant was treated in September 1930 and again in October 1935 for gonorrhea. He gave a history on these admissions of repeated attacks of anterior urethritis over a number of years.

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From the Department of Pathology, Washington University School of Medicine

1 Job, Levy and Morlot. Bull. Soc. d'obst. et de gynéc. 16 171, 1927

*Necropsy*—The body was that of a stillborn Negro girl. It weighed 500 Gm and measured 27 cm in length. The skin showed moderate maceration. There was no apparent inflammation of the conjunctivae, nor was there any other external evidence of infection. All the serous lined cavities contained dirty brown fluid, the product of early autolytic changes. The pleural and peritoneal surfaces were discolored but smooth and free from exudate. The visceral and the parietal pericardium were thickened and adherent. When these were pulled apart there was disclosed between them a sticky, adhesive exudate. The heart weighed 11 Gm and showed no abnormalities of development. The endocardium showed no gross lesions. The lungs were unexpanded. The abdominal viscera showed no gross abnormalities.

On microscopic examination the visceral and parietal pericardial surfaces were found covered with a thick layer of granulation tissue. This tissue was densely infiltrated by cells, most of them polymorphonuclear leukocytes. Between the thickened visceral and parietal pericardium was a thick exudate. It was formed of fibrin, large numbers of polymorphonuclear leukocytes and a few red cells and lymphocytes. In the outer layers of the myocardium the small capillary vessels were dilated. There was no inflammation of the endocardium.

The only other organs showing abnormal microscopic changes were the stomach and duodenum. In the lumen of these organs there was an exudate composed of desquamated epithelial cells, mucus and numbers of polymorphonuclear leukocytes. The mucosal lining was intact. The small capillaries of the submucosa were congested, and this layer of the wall was infiltrated with lymphocytes. The subserosal connective tissue was thickened and edematous. It was infiltrated by lymphocytes and mononuclear cells. In the thickened connective tissue were numerous large, dilated lymphatics, which contained masses of polymorphonuclear leukocytes.

Gram stains (Goodpasture-MacCallum method) of sections of pericardium and stomach were made, but no bacteria could be demonstrated. Acid-fast and Levaditi stains were made, but careful search failed to reveal any acid-fast bacilli or spirochetes. The microscopic study of the tissue failed to show any evidences of syphilis. No cultures were taken.

#### COMMENT

This was a case of organizing purulent pericarditis occurring in a stillborn Negro girl of approximately twenty-two weeks' gestation. The only other finding of note was the thickening and inflammatory infiltration of the subperitoneal connective tissue plus the presence in the cavity of the stomach and of the duodenum of acute inflammatory cells. The etiologic agent in the production of this acute inflammation cannot be demonstrated. Of particular interest is the positive history of gonorrhea in both mother and father.

The mother had endocervicitis with a profuse vaginal discharge during her pregnancy. She had a 4 plus complement fixation reaction to the gonococcus. Bacterial organisms could not be demonstrated in the fetal tissue even in the areas of acute inflammation. The possibility of connecting the fetal inflammation with the chronic gonorrheal infection in the mother is almost inescapable. The mode of such an infection is not clear. The placenta was not reported as offering any evidence of inflammation, nor was the amniotic fluid apparently abnormal. We found, however, acute inflammatory cells in the stomach and duodenum.

and thickening and inflammatory infiltration of the subperitoneal tissues. In these thickened tissues were dilated lymphatics filled with polymorphonuclear leukocytes. A possible supposition is that the amniotic fluid was infected (perhaps by direct extension through the membrane, from the cervical canal) and that this infected fluid was swallowed, the fetal infection resulting from absorption of organisms from the gastrointestinal tract. The most prominent localization of the infection was in the pericardial sac, as evidenced by the acute purulent pericarditis.

Although the question of fetal septicemia has received considerable study, little has been reported regarding gonococcic fetal infections. In reports on large series of fetal deaths one does not find the gonococcus mentioned as an etiologic agent. Slobozianu and Herscovici<sup>2</sup> reviewed the literature on gonococcic fetal infections and from it collected 5 cases of gonococcic arthritis in the newborn, to which they added 3 cases of their own. In most of these cases the mother had active gonorrheal arthritis during pregnancy. In several of the cases cited arthritis did not appear in the infant until several days after delivery. The authors concluded that since there was no ophthalmia or similar evidence of external infection, the arthritis in these infants was merely the result of an infection contracted during fetal life. They expressed the belief that although the mother may not give evidence of having active septicemia during pregnancy, transient phases may occur during which organisms penetrate the placenta and enter the fetal circulation.

#### SUMMARY

Organizing purulent pericarditis in a stillborn Negro girl of 22 weeks' gestation is reported. In view of the positive history of gonorrhea in the mother and father, it is suggested that the fetal inflammation may have been of gonococcic origin.

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2 Slobozianu, H., and Herscovici, P. *Gynec et obst* 28 601, 1933

## Obituaries

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ALFRED STENGEL, M D

1868-1939

Alfred Stengel, M D , D Sc , LL D (Lafayette and Pennsylvania), died April 10, 1939, in the seventy-first year of his life

Dr Stengel was born in Pittsburgh on Nov 3, 1868. He received the degree of Doctor of Medicine at the University of Pennsylvania on May 1, 1889, after which he served as intern at the Philadelphia General Hospital until Nov 1, 1890, the last three months of the time being an extra service in the division of internal medicine. The following year he began his career at the University of Pennsylvania in the divisions of embryology and pathology and then became assistant to Dr William Pepper and clinical clerk to him. This appointment was succeeded by that of instructor in clinical medicine, which position he held until 1899, he then became professor of clinical medicine, a grade that he held until 1911, when he became a full professor of medicine. He retired from this role in 1937, after a service of twenty-six years, to devote his major time to the duties of vice president in charge of medical affairs in the cabinet of the president of the university, Thomas S. Gates. This position, established in 1931, was the first of its kind, a part of the new system originated by Mr Gates. During the eight years of his devotion to the duties of this office, Dr Stengel was most active in broadening the relationships of the various schools that touch on biology and was able to associate closely the schools of medicine, veterinary medicine and biology and the Wistar Institute. The graduate school of medicine, already started, but with its greatest activity at a distance from the university, was by Dr Stengel brought nearer physically and more closely affiliated with the splendid teaching facilities of the parent institution. In 1937 he was selected by the state of Pennsylvania to be the manager of the school of animal pathology at the university, which operates with the aforementioned divisions in collaboration with the state.

While Dr Stengel's life was devoted in a very large part to the University of Pennsylvania, he held many other positions not directly connected with the university—positions which helped especially in the early education of the man in his chosen field. His early interest in pathology, which will be indicated by a later consideration of the literature which he produced is shown by his close connection with the Philadelphia Pathological Society for many years, from 1891, and

the fact that in 1893 he was elected pathologist to the German Hospital, now the Lankenau, a post which he kept until 1897

In 1895 Dr Stengel was appointed assistant director and in 1898 full director of the William Pepper Laboratory of Clinical Medicine, a position which he retained until 1911. He was physician to the Pennsylvania Hospital from 1900 until he retired from active duty, becoming consulting physician in 1925. He was professor of medicine at the Womens' Medical College for two years, from 1896 to 1898,



ALFRED STENGEL, M D  
1868-1939

and chief in medicine at the Howard Hospital from 1895 to 1897. He was also consulting physician to several other hospitals. He was a member of the American Philosophical Society from 1901 until his death. The Philadelphia College of Physicians elected Dr Stengel as vice president in 1931, and from 1934 to 1936, inclusive, he served as president.

Notable, particularly in the last twenty years of his life, is the number of national committees, such as the National Research Council, on which he was invited to serve. He was president of the American

College of Physicians in 1925, 1926 and 1927, and he was on the board for the reconstruction of its constitution. He was on the board of the Wistar Institute and became its president in 1937, succeeding Effingham B. Morris. There can be added to this formidable list of services rendered by Dr. Stengel a considerable number of less conspicuous connections, but those mentioned serve to illustrate how often he was sought and how broad were his interests, how energetic his services and how capable his judgment.

As one begins to consider his professional life, one is struck by the fact that before his graduation, in 1889, and therefore before there was practically any literary activity by students, he wrote a paper on albuminuria, and during his internship at the Philadelphia General Hospital he wrote three more. The first three were for students and interns, while the last, on acute dysentery and *Amoeba coli*, was published in the *Medical News* of November 1890.

There is a strong suggestion, however, that Dr. Stengel had an early active interest in pediatrics, since he served, beginning in 1896, for three years as physician to the Children's Hospital. Some of his writings, particularly in Starr's "System of the Diseases of Children," indicate this inclination.

Dr. Stengel's interest in pathology probably was started and strengthened by his association with Juan Guiteras and Allen J. Smith. He wrote the notes for publication on Guiteras' lectures in 1891. From then on appeared a series of articles on the blood, nephritis and diseases of the heart and lungs and many reviews that indicated the pathologic analysis to which he subjected all his reading. He participated actively with Dr. William Pepper in the preparation of the latter's "System of Medicine." In 1898 he issued a "Textbook of Pathology" that ran into eight editions. In the preparation of the last three of these I had the privilege to be associated with him. In 1901 he assumed the American editorship of "Nothnagel's System of Medicine," which came out in twelve volumes. The literary contributions of Dr. Stengel ran to 179 accepted and published titles, of which only 10 were not strictly medical. Dr. Stengel was on the editorial board of the *ARCHIVES OF PATHOLOGY* from the start of its publication.

Dr. Stengel's development seems to have been coincident with the early changes of the influence of organic pathology in eradicating empiricism from the leading medical minds of this country. The teachings of Virchow, Cohnheim, Claude Bernard and others were reaching this country with great force. Morbid anatomy was started in this country one hundred years ago by Horner and continued in 1839 by Samuel Gross, both surgeons. It was not until 1885 that Delafield presented a separate and systematic study of pathology. However, with William Pepper, Flint and others of the American school there came a demand that pathology be thought of first in the

conception of human illness, and under this influence, in the years between his graduation and 1898, Dr Stengel pressed himself through a discipline that led up to the publication of his book on the subject. Previous books offered cases in exemplification or discussed abstractly the possibilities of cause and effect of the morbid change. Dr Stengel pictured the etiologic factors, the objective findings and the possible effects in a manner that forced the reader to associate the anatomic and physiologic changes with the actual clinical status of the patient—the systematic and paragraphic method practiced by the German school. This method continued to be evident throughout all his editions and indeed was shown in his teaching. He was not in speaking or writing discursive, but he followed a line of stern logical history, without dogmatism or didactics, building up a structure and then asking why and how the morbid processes were responsible and what they would mean for the future. He taught with strict objectivism, never losing the reasoning that was based on facts. He was not an abstract research worker, but he used his early knowledge of embryology and anatomy, for which he had a marvelous memory, and kept so abreast of modern physiology and chemistry that he had no difficulty in maintaining a constant balance in his dealing with a clinical problem. It is especially interesting that he could place the safest value on the details that made up the whole. He never lost sight of the woods for the trees, but a better judge of an oak never lived.

Between the early formative period and 1931, when he became vice president of the university, his major time was given to the practice of internal medicine and to his students. These men became better men because Dr Stengel taught them to be self critical.

Personally, Dr Stengel was of medium height or slightly less, active and definite in action, without loss of motion, of an alertness that showed itself more in his mind than in his body. Though his thought was rapid, measurement and balance were never absent. Dr Stengel's accessibility to all who had a worthy subject was amazing. He refused no one, and his advice, based on a broad experience of human activity, was always at the disposal of the worthy visitor. His psychological understanding seemed to extend everywhere. He employed it with amazing skill in the handling of patients, and the medical world knows that this is necessary to a great practitioner. And Dr Stengel was a great doctor of the sick. To what degree this may have been due to his personality, he gave credit to pathology. He said once to me that he hoped no man would ever treat him for pneumonia who had never held a pneumonic lung in his hand.

The world at large, and most deeply those who knew him, grieve at the loss of a man who, although he had reached the usual promise of three score and ten, appeared to have at least a decade of joy for his friends and of value to society.

HERBERT FOX

## Notes and News

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**University News, Promotions, Resignations, Appointments, Deaths, Etc**—The portrait of H G Wells painted by Wayman Adams was presented by friends of the pathologist to the University of Chicago on June 23. The portrait will hang in the lecture room of the department of pathology.

Honorary degrees have been conferred by Yale University on Hans Zinsser and E W Goodpasture. Dr Zinsser has received an honorary degree from Harvard University, also.

Maurice Biodie, National Research Council fellow in medicine 1933-1934, assistant director of laboratories in Providence Hospital, Detroit, died on May 9, 1939, aged 35 years.

**Anna Fuller Prize**—The first award of this prize goes to E L Kennaway, J W Cook and three other scientists at the Research Institute of the Royal Cancer Hospital of London, who will share \$7,500 equally for their work on the cancerigenic hydrocarbons of coal tar.

**Changes in Officers and in Board of Managers of Wistar Institute**—The Wistar Institute of Anatomy and Biology, Philadelphia, announces the election of Esmond R Long as president of the board of managers and of Alfred N Richards and William H DuBarry as members of the Board. Edmond J Fairis has been appointed executive director of the institute.

**Society News**—The following officers were elected at the annual meeting of the American Society of Clinical Pathologists in St Louis: president-elect, A V St George, vice president, C L Klenk, and secretary-treasurer, A S Giordano.

**Finney-Howell Research Foundation**—Eight fellowships for cancer research have been renewed and six new fellowships have been awarded, also two grants-in-aid. Applications for 1940 awards must be received before January 1 next, addressed to the secretary, Dr William A Fisher, 1211 Cathedral Street, Baltimore.

**Medical Examiner for Maryland**—In Maryland a law has been enacted, in accordance with which the coroner system will be replaced by the medical examiner system. It provides for a chief medical examiner and two assistants for the city and county of Baltimore, all on a full time, salaried basis. In each outlying county there will be a deputy examiner, whose duty it will be to make the preliminary medical investigation. If further investigation is necessary, such as postmortem or toxicologic examination, it is to be made by the medical examiner of Baltimore and the cost charged against the county for which the work is done. All appointments are to be made by a nonpaid, nonpolitical commission composed of the professor of pathology of the Johns Hopkins University, the professor of pathology of the University of Maryland, the city health commissioner of Baltimore, the state health commissioner and the state attorney general.



# Abstracts from Current Literature

TO SAVE SPACE THE ORIGINAL TITLES OF ABSTRACTED ARTICLES SOMETIMES ARE SHORTENED

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## Experimental Pathology and Pathologic Physiology

VIABILITY IN "DEVASCULARIZED" LYMPH NODES R L HOLMAN and E B SEIF,  
Am J Path **14** 463, 1938

Popliteal lymph nodes of dogs when replaced in the popliteal space after complete severance of all vascular and lymphatic connections rapidly undergo massive necrosis. These nodes usually become infected and may slough out. When, however, all vascular connections are severed but one or more afferent and one or more efferent lymphatic channels remain intact, infection does not ensue, and the nodes remain viable. Chemical analyses of lymph flowing to and from these "devascularized" nodes show a sharp drop in reducing substance, bound carbon dioxide and carbon dioxide-combining power in the lymph during its passage through the node, and indicate that anaerobic glycolysis is one of the metabolic processes taking place in the viable node. These observations imply a nutritive function on the part of lymph which, so far as the authors have determined, has not been demonstrated in vivo in mammals.

FROM AUTHORS' SUMMARY

RENAL FUNCTION AS AFFECTED BY AN EXPERIMENTAL LESION OF ONE KIDNEY  
T F NICHOLSON, R W I URQUHART and D L SELBY, J Exper Med  
**68** 439, 1938

A method is described for the production of nephrosis in one kidney of an experimental animal. The normal kidney in the same animal is available as a control. The nephrosis, produced by injecting 7.5 per cent sodium tartrate, is limited to the proximal convoluted tubule. There is no histologic evidence of glomerular damage. The tubular damage results in a disturbance of the excretion of water and chloride as well as in a decrease in the clearance of urea, xylose, inulin, creatinine and phenol red, and is observed under conditions of no diuresis, sugar diuresis and salt diuresis. In the cells of the proximal tubule of the damaged kidney one finds excreted ferrocyanide. This substance is not present in the corresponding cells of the normal kidney. This observation is taken to be evidence that there has been back diffusion of ferrocyanide through the damaged cells. The decrease in clearance of creatinine and inulin, which in the dog represent glomerular filtrate, may therefore be explained by back diffusion and as not necessarily due to glomerular change. The clearance of phenol red from the damaged kidney is diminished in relation to the clearance of inulin. This is further evidence of depression in tubular activity.

FROM AUTHORS' SUMMARY

HYPOPHYSECTOMY IN THE RAT A C CROOKE and J R GILMOUR, J Path & Bact **47** 525, 1938

A series of 114 immature rats were subjected to hypophysectomy and killed from two to one hundred and two days afterward. Complete hypophysectomy resulted in almost total arrest of increase of body weight and in conspicuous atrophy of the adrenals and testicles and a very slight defect in the weight of the thyroid, these glandular changes being approximately maximal in fourteen, twenty-eight and seven days, respectively. When hypophysectomy was incomplete, regeneration of the anterior lobe in some cases caused, in time, disappearance of

these changes to a variable degree. Loss of body weight sometimes persisted for fourteen to twenty-eight days. Abnormal obesity was associated with accidental injury penetrating the floor of the third ventricle in 5 rats but was absent from 6 rats with apparently similar injuries. One of these obese rats showed, like the others, changes in the adrenals, testicles and thyroid similar to those in completely hypophysectomized animals although two relatively large remnants of the anterior lobe of the pituitary were found microscopically. These remnants were abnormal in showing complete absence of chromophil cells. After complete hypophysectomy microscopic examination showed in the testicles reduction of spermatocytes by fatty degeneration and necrosis, cessation of spermatogenesis, proliferation of basal cells and reduction in the number and size of interstitial cells. In the adrenals the cells of the inner layer of the cortex, particularly of its inner part, were greatly reduced in number by degeneration, partly fatty, and by necrosis, lymphocytic infiltration and pigmentation were slightly increased in the inner part of the inner cortical layer, lipid was diminished in the inner cortical layer. In the thyroid the signs of active normal secretion and excretion were replaced by those of colloid storage. The degree of these glandular changes at different intervals after operation is described.

FROM AUTHORS' SUMMARY

CHANGES IN THE ADRENALS IN EXPERIMENTAL HYPERTHYROIDISM G HABAN, Beitr z path Anat u z allg Path **101** 45, 1938

Thyroxin was administered to rabbits, cats and guinea pigs over a period of several months. The result was a marked increase in the size and weight of the adrenals. The increase was most pronounced in the cortex, particularly in the zona fasciculata, where an accumulation of lipoids, especially of the anisotropic type, was found. It remains to determine whether the enlargement of the adrenals is the result of hyperthyroidism.

R J LEBOWICH

### Pathologic Anatomy

VASCULARIZATION OF ATHEROSCLEROTIC LESIONS T LEARY, Am Heart J **16** 549, 1938

It must be concluded that the normal intima is not vascularized, and that blood vessels are found in the intima only in connection with vascular lesions in which repair has taken place. Since the vessels arise as the result of repair processes, i. e., since the lesions are advanced atherosclerotic lesions, the hemorrhages from such vessels must be late phenomena and therefore are not of etiologic importance. Cholesterol found in atherosclerotic lesions is transported to the site in macrophages or as free or ester cholesterol in the blood, it does not arise locally except, perhaps, in small amounts. The laws of nutrition and growth still hold in atherosclerotic lesions.

FROM AUTHOR'S SUMMARY

SUBACUTE AND CHRONIC GLOMERULONEPHRITIS E T BELL, Am J Path **14** 691, 1938

Bell studied 181 cases of glomerulonephritis, the condition in 16 was classified as subacute, in 8 as latent chronic, in 117 as chronic azotemic and in 40 as chronic hydropic. In subacute glomerulonephritis the kidneys are not contracted. There is widespread severe glomerular obstruction with well advanced uniform tubular atrophy. There are few hyaline glomeruli. Altogether, 124 cases of chronic azotemic glomerulonephritis were included. Eight cases of latent chronic glomerulonephritis are described, in which death was due to intercurrent disease. Only 1 such case has been reported previously. In such cases there are only a few hyaline glomeruli, and there is little or no tubular atrophy. The glomeruli are all damaged to some degree, their lobules showing hyaline central portions and peripheral capillaries. Thirty cases of chronic azotemic glomerulonephritis are

reported in which there was a history of an initial acute attack. The total duration varied from one and a half years to twenty-six years, with an average duration of ten years. In 15 of the 30 cases the acute attack was followed by a latent chronic stage, varying from one year to twenty-four and a half years in length, in the remaining 15 cases the acute state passed directly into active chronic nephritis. In 30 per cent of the cases the systolic blood pressure was 200 mm of mercury or higher. There was some degree of chronic passive congestion of the liver in nearly one half of the cases, indicating some degree of heart failure, but there was no evidence that heart failure was ever more than a contributing cause of death, since all the patients had uremia. Heart failure may occasionally have been a contributory cause of edema. Only a single patient had a history of apoplexy. Only a single patient had an attack of coronary sclerosis. Retinitis was found in 35 of 46 patients whose eyegrounds were examined. There is a definite relation between high blood pressure and retinitis. There is no relation between the weight of the kidneys and the duration of the disease or the height of the recorded blood pressure. The kidneys were occasionally of normal size or even enlarged in the terminal stages. Large kidneys contained a high proportion of injured glomeruli with moderately atrophic tubules, while small kidneys consisted largely of hyaline glomeruli with extremely atrophic tubules. Forty cases of hydropic glomerulonephritis (lipoid nephrosis) are reported. In 6 of these the glomerular structure was that of chronic proliferative glomerulonephritis, in 9 others there was a mixture of proliferative glomerulonephritis, and in 9 others there was a mixture of proliferative and membranous glomerular lesions, with the latter in great preponderance. In the remaining 25 cases there were no proliferative lesions. In 6 cases in which young children were concerned there were no visible changes in the glomerular capillaries, and in 3 other cases involving children there were only focal membranous lesions. With 1 exception, diffuse thickening of the basement membranes was present in all persons over 12 years of age. In 4 cases membranous glomerulitis produced such extensive narrowing of the glomerular capillaries that uremia developed. When a patient with pure lipoid nephrosis acquires hypertension and uremia, no new disease is superimposed—there is merely progressive thickening of the basement membranes. Nephrosis is a form of glomerulonephritis in which the glomerular capillaries remain open and allow the blood proteins to escape into the urine. In proliferative glomerulonephritis the capillary lesions are nearly always obstructive in type.

FROM AUTHOR'S SUMMARY

ACUTE HEMATOGENOUS INTERSTITIAL NEPHRITIS P. KIMMELSTIEL, *Am J Path*  
14 737, 1938

Six cases of acute hematogenous interstitial nephritis are presented, in all of which there was more or less marked isosthenuric oliguria or anuria, with a rise of nonprotein nitrogen in the blood. Twelve other cases of interstitial nephritis, in all of which the condition was found incidentally at autopsy, are included. The morphologic characteristics of this lesion are described. It is emphasized that even in the early stage the interstitial exudate contains plasma cells, lymphocytes, eosinophils and monocytes in addition to polymorphonuclear leukocytes. A distinction between focal and diffuse interstitial nephritis should not be made. The conditions causing interstitial nephritis are listed. In addition to the infectious diseases and septicemia, it is found to follow conditions associated with hemolysis, in particular transfusions of incompatible blood. It is also found in the hepatorenal syndrome associated with infectious and noninfectious injuries of the liver. The correlation of functional disturbances with interstitial nephritis is discussed. It is concluded that the anuria does not result from renal edema by pressure on the tubular apparatus, neither is it caused by blockage of tubules if associated with hemoglobinuria. General or local renal circulatory disturbances are held to be the most likely cause of oliguria and of lack of power of concentration. Hema-

togenous interstitial nephritis is regarded as an allergic hyperergic reaction to foreign proteins or protein split products coincidental rather than causatively connected with hyposthenuric oliguria and anuric uremia

FROM AUTHOR'S SUMMARY

THE SILICOTIC NODULE J WALSH, *Am Rev Tuberc* **38** 363, 1938

Anthracosilicotic dust within the tissues of the lung is always found in cells until after necrosis sets in. These cells are carried in lymph channels to intrapulmonary and hilar nodes. The pulmonary fibroanthracosilicotic nodule begins with thickening of the interstitial tissue around lymphatics, development of fibrous tissue in alveolar walls and destruction of the elastica, which allows the alveoli to collapse. The typical single nodule is smaller than a millet seed and is made up principally of collapsed and coalesced alveolar walls. Later occur hyalinization and necrosis, beginning in the interior. A large nodule is always conglomerate and is made up of the coalescence of innumerable small ones. The silicotic nodules in lymph nodes are also described and differentiated from the pulmonary nodules.

H J CORPER

CHANGES IN THE LIVER IN CHRONIC PASSIVE CONGESTION E W BOLAND and F A WILLIUS, *Arch Int Med* **62** 723, 1938

The usual histologic picture of the liver in prolonged or recurrent congestive heart failure is that of atrophy or necrosis or both at the centers of lobules. Condensation of reticulum and thickening may or may not be present. The presence of condensation of reticulum alone does not warrant the use of the term "cardiac cirrhosis." True cirrhosis does develop in the course of congestive heart failure, but it does so rarely. No definite criteria were elicited from this study whereby the development or the presence of cardiac cirrhosis can be recognized clinically.

FROM AUTHORS' SUMMARY

BIOPSY STUDIES OF CEREBRAL CHANGES IN SCHIZOPHRENIA AND MANIC DEPRESSIVE PSYCHOSIS A R ELVIDGE and G E REED, *Arch Neurol & Psychiat* **40** 227, 1938

Elvidge and Reed investigated, with one of Hortega's silver staining methods, the oligodendroglia in pieces of brain removed during life from patients with schizophrenia, manic depressive psychosis and so-called toxic encephalitis. Two types of swollen oligodendroglia cells were observed. One type contained a nucleus of normal size, in the other type the nucleus was shrunken, pyknotic and vacuolated, and the cytoplasm was increased. The swelling of the oligodendroglia cells remained for a long time, as repetition of biopsies at the end of one or two years showed, i. e., there may be acute and chronic processes present.

G B HASSIN

HERNIATION OF THE BRAIN NOT HERETOFORE DESCRIBED I FINKELMAN, *Arch Neurol & Psychiat* **40** 803, 1938

Finkelman describes two forms of herniation of the brain not hitherto described in association with tumor or abscess, aneurysm, subdural hematoma and other intracerebral lesions. In one form, which occurred in 80 per cent of the cases of tumor of the frontal lobe, there was bulging of the frontal lobe over the sphenoid ridge and pressure on the tip of the temporal lobe, often on the side opposite the site of the tumor. Occasionally it was bilateral and was associated with hydrocephalus and papilledema. The other form of herniation concerned the gyrus rectus. The posterior part of this gyrus was herniated over the edge of the limbus sphenoidalis, compressed the optic nerve and was practically always associated with choked disk.

G B HASSIN

HUMAN ELLIPTIC RED CORPUSCLES A L FIORMAN and M M WINTROBE, Bull Johns Hopkins Hosp 63 209, 1938

The term "elliptic erythrocyte trait" is defined Eleven instances of the trait in three families are recorded A study has been made of the occurrence of these cells in the absence of the hereditary trait In 89 per cent of a series of 62 non-anemic persons, between 1 and 15 per cent of the cells were elliptic In a series of 100 consecutive patients with anemia, 98 per cent showed many of these cells, and in 12 per cent of these more than 25 per cent of the cells were found elliptic Elliptic cells have been found to be more commonly associated with macrocytic than with other types of anemia In cases of macrocytic anemia these cells seemed to become somewhat more common as the anemia became more severe, but a well defined correlation could not be demonstrated The anomalous shape seems to be a property of the cells This shape is characteristic of the mature rather than of the immature red corpuscles of this type

FROM AUTHORS' SUMMARY

INFARCTION OF BONES IN CAISSON DISEASE S C KAHLSTROM, C C BURTON and D B Phemister, Surg, Gynec & Obst 68 129, 1939

Although destruction of joints and bones has been recognized as one of the manifestations of caisson disease since 1911, the lesions have never been described pathologically In the 4 cases now reported the condition was of long standing, and there was evidence of multiple infarction of long bones In 1 case the diagnosis was confirmed by autopsy and in another by biopsy When the necrotic bone was situated in the epiphyses and bordered on joints, varying amounts of collapse of the weight-bearing portions, invasion and replacement by new bone and calcification of nonsubstituted portions were noted Articular cartilage overlying affected areas was replaced by fibrocartilage, and more or less extensive arthritis deformans was established Collapse did not occur when the necrotic bone was situated in the diaphyses or in the epiphyses away from the articular surfaces On the other hand, there was evidence of some invasion and replacement by new bone and probably complete replacement of some of the smaller foci

Uncertainty prevails as to whether the necrosis was produced by nitrogen gas obstruction of end arteries or by direct pressure on blood vessels and other tissues after liberation of nitrogen from solution in the fat of the bone marrow or in some other unexplained way The facts that long bones, which are rich in fatty marrow, were the only ones involved, that fat absorbs relatively large amounts of nitrogen and that nitrogen bubbles would be absorbed slowly from the marrow favor the view of direct pressure of the gas on vessels and other tissues within the bone On the other hand, the extensive involvement of the diaphyses in some cases without lesions in the epiphyses, especially of the head of the femur, without involvement of the diaphyses or without continuity of affected regions in epiphysis and diaphysis are points in favor of embolism by nitrogen gas or some other form of intravascular obstruction Experimental air embolism of the lower limbs of dogs failed to produce bone necrosis

FROM AUTHORS' SUMMARY (WARRREN C HUNTER)

EPITUBERCULOSIS R H FISH and W PAGEL, J Path & Bact 47 593, 1938

A survey of the literature on "epituberculosis" is made, and the observations at necropsy in 3 new cases are recorded The process appears to be related to a primary caseous pulmonary focus but may also be observed in postprimary tuberculosis of adults It is concluded that in cases in which a condition has been clinically diagnosed as "epituberculosis" a noncaseating tuberculous tissue reaction may be found and may be regarded as the normal response of tuberculoallergic lung tissue to contact with material containing tuberculous protein but few live

tubercle bacilli This type of lesion should be distinguished from collapse due to external bronchial compression, which may give rise to a comparable clinical picture However, mixed cases are common FROM AUTHORS' SUMMARY

THE THYROID GLAND OF THE CRETIN AT BIRTH J EUGSTER, Beitr z path Anat u z allg Path **100** 392, 1938

Prompted by the almost complete absence of knowledge of the anatomic state of the thyroid gland of the cretin at birth, Eugster investigated 4 such glands The findings were not uniform, 2 showing a pronounced diffuse parenchymatous struma, and a third distinct atrophy and sclerosis In the fourth there was a slight, probably goitrous enlargement The difficulty of establishing a clinical diagnosis of cretinism at birth is discussed in the light of the pathologic changes

R J LEBOWICH

### Microbiology and Parasitology

THE PRESENT INCIDENCE OF TRICHINELLA SPIRALIS IN MAN AS DETERMINED BY A STUDY OF ONE THOUSAND AND SIXTY UNSELECTED AUTOPSIES IN ST LOUIS HOSPITALS T B POTE, Am J M Sc **197** 47, 1939

Muscle tissues obtained at 1,060 autopsies were trichinous to the extent of 15.4 per cent There had been no symptoms suggesting trichinella infection In 95 per cent of the specimens the trichinellas were dead, calcified and more or less disintegrated, in 5 per cent they were demonstrated to be alive by feeding the infected material to rats and recovering live larvae Very heavy infections were to be observed in cases in which the host gave a history of having always enjoyed good health In a patient dying at the age of 84 as the result of an accident autopsy did not show that the infection had contributed to the cause of death

In a series of 1,500 hog carcasses examined for trichinella, 0.8 per cent were found to be infected, an observation which gives rise to the question whether man is getting all of his trichinella infection from pork It may be concluded that man's infection comes from pork derived from the small packing plants, which are not supervised by meat inspection services It is to be noted that the infections that sporadically occur are from pork from establishments not under federal inspection

It can be definitely concluded that *Trichinella spiralis* infection of man is not a serious lethal factor in Missouri, since in 163 instances of infection it was not held to have been the cause of death or to have contributed to the cause of death

FROM AUTHOR'S SUMMARY

INTOXICATION AND RESISTANCE TO INFECTION K L PICKREIL, Bull Johns Hopkins Hosp **63** 238, 1938

Alcoholic intoxication maintained at the point of stupor destroys resistance to pneumococcal infection in the rabbit Even animals rendered highly immune by intravenous injections of antipneumococcus serum were deprived of their immunity The loss of resistance to the infection appears to be due to the fact that intoxication profoundly inhibits the vascular inflammatory response as long as it is maintained, and in the absence of capillary dilatation and emigration of the leukocytes, leukocytic margination at the site of infection is negligible, and the bacteria therefore proliferate uninterruptedly Similar experiments show that ether or avertin anesthesia has as marked an inhibitory effect on the inflammatory response and produces as marked a loss of resistance to infection

FROM AUTHOR'S SUMMARY

DETECTION OF THE VIRUS OF POLIOMYELITIS S D KRAMER, B HOSKWITH and  
L H GROSSMAN, J Exper Med 69 49, 1939

Five strains of the virus of poliomyelitis were recovered from nasal washings and feces. Four strains were of human origin, the fifth strain came from a monkey put to death at the height of the disease. Of the human strains, the first was isolated from the feces of a 14 year old child seven days after the onset of illness. The second strain was from the nasal washings of a 6½ year old child five days after the onset of illness. One of these strains was obtained from nasopharyngeal washings and the other from feces. The single monkey strain was isolated from the upper intestinal segment, and this appears to be the only instance of isolation of a strain of this virus from such a source recorded in the literature. The authors state that the detection of the virus in the nasal washings of 2 additional patients who were convalescent lends further support to the belief that the virus of poliomyelitis is spread by human contact. Furthermore, the recovery of the virus from the gastrointestinal tract with as great or greater frequency than from the upper respiratory tract need not, it appears to these authors, alter the present concept of the mode of entrance of the virus into the body, namely, by way of the upper respiratory tract. If the presence of the virus in the upper respiratory tract is conceded, the passage of nasal and oral secretions into the gastrointestinal tract by reflex swallowing would serve to explain the presence of the virus in this tract. It might be further predicated that since the gastrointestinal tract functions as a temporary reservoir for secretions from the upper respiratory tract, the intestine should, after a time, contain the virus in higher concentration than any single sample of secretion obtained from the upper respiratory tract by nasal washing. Failures to detect the virus in the gastrointestinal tract are perhaps more indicative of the inadequacy of the procedures for the detection of the virus than of the absence of the virus. The recovery of the virus from the feces seven and nine days after the onset of illness takes on added significance. It indicates first that the virus withstands the gastric acidity which under normal physiologic conditions tends to keep gastric contents relatively free from bacteria. It further suggests that improper disposal of feces from patients with poliomyelitis may have serious consequences for public health, particularly in smaller communities, where the inadequate disposal of sewage may result in contamination of surrounding beaches or even local water systems.

FROM AUTHORS' SUMMARY

\*STUDIES IN THE METABOLISM OF COCCIDIOIDES IMMITIS (STILES) R A STEWART  
and K F MEYER, J Infect Dis 63 196, 1938

The resistance of *Coccidioides* to desiccation may be an important factor in the dissemination of the organism in the dry, warm climate of the San Joaquin Valley, in California. Under conditions of drying the chlamydospore is formed, and it is apparently not possible to infect animals in the absence of this spore.

A synthetic medium has been devised which possesses selective properties of value in the isolation of *Coccidioides* from the soil and a wide variety of clinical materials.

The metabolism of *Coccidioides immitis* and *Blastomyces dermatitidis* did not present any significant difference in either a protein-rich medium or a synthetic medium.

Contrary to the literature, dextrose is assimilated by both *Coccidioides* and *Blastomyces*. This throws doubt on the accuracy of the statements relative to consumption of sugar based on changes in the  $p_H$  of a medium. Changes in the  $p_H$  occur independently of the consumption of dextrose and are to be explained by variations in the consumption of other constituents in the medium.

The metabolism of *C. immitis* and *B. dermatitidis* differs from the metabolism of bacteria and certain protozoa in that the consumption of dextrose does not suppress the production of ammonia. The assimilation of dextrose favors the production and the assimilation of ammonia.

Fungi are biologic entities or individualists and are not always amenable to the principles established by bacteriologic procedures—they require their own type of medium. The medium should be chemically defined whenever possible, so that morphologic and physiologic characteristics may be referred to an environment that can be duplicated anywhere. This type of medium offers the best "common denominator" for the correlation of results reported by widely scattered investigators.

FROM AUTHORS' SUMMARY

EPIDEMIC INFLUENZA VIRUS T. P. MAGILL and F. T. JUN. Brit. J. Exper. Path. **19** 273 and 284, 1938

Cross neutralization tests performed with 24 strains of influenza virus are reported, and the significance of the findings is discussed. Although marked differences between some strains and striking similarities between others permits rough grouping, the characteristics of one group tend to merge into those of another. The strains which most closely resemble one another are in general those from the same epidemic of influenza, but serologically different strains may be recovered from the same epidemic. The specific effect of a given serum can be demonstrated by its action in tissue cultures as well as by the usual mouse protection test. So far as it has been possible to determine, the antigenic differences between strains have not resulted from variations occurring in the course of animal passage or artificial cultivation. It is suggested that the structure of the virus of epidemic influenza is a mosaic of antigens, numerous strains containing the same antigens arranged in such a way that each strain or group of strains is characterized by its own peculiar antigenic pattern. It is further suggested that all strains may not contain all the antigenic components of all other strains.

Cross immunity tests were carried out with 16 strains of epidemic influenza virus and 1 strain of swine influenza virus in actively immunized mice. It was found that antigenic differences in strains such as those demonstrated by serologic tests in the preceding paper are reflected in a lack of cross immunity. In general, strains obtained in the same year tend to be closely related immunologically, although individual differences exist. While quantitative factors are undoubtedly involved in the problem of cross immunity between strains, qualitative differences can be shown in strains of equal virulence. The differences scarcely seem to warrant a designation of types, since most strains of human origin are closely related. The importance of the serologic and immunologic differences between strains from the point of view of the problems of epidemiology and prophylaxis is discussed.

FROM AUTHORS' SUMMARY

THE STRUCTURE OF "ROUGH" AND "SMOOTH" COLONIES K. A. BISSET, J. Path. & Bact. **47** 223, 1938

The structure of a morphologically rough colony of a bacillus is essentially similar to that of the medusa head colony of the anthrax bacillus. The bacilli lie close together in threads. The bacilli composing a smooth colony are all separate and show no characteristic arrangement. Between these extremes are a number of intermediate forms. These variations are paralleled by similar forms found among the streptococci. The usually described SR variations appears to consist of two separate changes, one affecting the morphologic appearance of the individual organism, the other connected solely with the nature of its surface material. These may occur separately, though a considerable degree of relationship appears to exist. The structure of a colony depends ultimately on physical factors and varies mainly with the degree of attachment shown by the component organisms.

FROM AUTHOR'S SUMMARY



SURVIVAL OF RICKETTSIA PROWAZEKI IN LICE J STARZYK Arch Inst Pasteur de Tunis **27** 263, 1938

Typhus rickettsias were found to survive as long as six days in human serum diluted 1:1 and maintained at 5 to 7 C, and a lesser period under other conditions, as tested by the percentage of lice infected. Also when kept cold and dried under a vacuum they survived six days. The results not only indicate a useful point in preserving rickettsias for use in studies or in the production of vaccines or in shipping but may have epidemiologic significance. The survival of the infective agent in clothing and on premises for some days after the death of infected lice raises new questions with regard to dissemination and control of typhus.

M S MARSHAL

## Tumors

CANCER IN RATS TREATED WITH OESTRONE C S McEUEEN, Am J Cancer **34** 184, 1938

Rats were treated for long periods with an estrogen (theelin, or estione), usually in conjunction with various forms of local irritation. The occurrence of tumors histologically diagnosed as malignant was found to be more frequent in these animals than in controls or in rats of the same colony subjected to other procedures, used for breeding or set aside without treatment.

FROM AUTHOR'S SUMMARY

ACTION OF COLCHICINE ON A TRANSPLANTED MALIGNANT NEOPLASM F J LITS, A KIRSCHBAUM and L C STRONG, Am J Cancer **34** 196, 1938

A malignant transplanted lymphoid tumor was subjected to a subcutaneous injection of 0.025 mg of colchicine in 0.1 cc of distilled water every three days, each injection being made at some distance from the tumor. By this treatment the tumor was caused to regress, and the animals survived fifty and one-half days after the implantation of the tumor, as compared with thirty-one and one-half days for untreated controls (13 controls and 14 test animals). Histologic studies showed that the regression was due to repeated so-called "caryoclastic shocks", the lymphocytes of the tumor became pyknotic and died. The reticular cell elements of the tumor were most resistant to treatment by colchicine. There were also some foci of resistant lymphocytes. Tumors which recurred seemed to arise by proliferation of the reticular cells of the regressed growth. Both the lymphoid and the reticular cells of the lymphosarcomatous growth studied may be considered malignant. A difference in susceptibility to colchicine exists between normal and malignant lymphocytes, the malignant cells being much more susceptible. Normal lymphocytes of the thymus are destroyed quite readily by the drug, in contrast to the more resistant lymphocytes of the spleen and lymph nodes.

FROM AUTHORS' SUMMARY

STRUCTURAL DEVELOPMENT OF GLIOMA H J SCHERER Am J Cancer **34** 333, 1938

A systematic study of the structural evolution in 100 gliomas has been made by means of large pyroxylin (celloidin) sections taken in multiple planes and including the whole tumor with its surrounding tissue. Glioma structures depend on the preexisting nerve structures (secondary structures) or on primary architectural properties inherent in the glioma cells (proper structures). The structure of a glioma may be influenced, also, by connective tissue which invades the tumor (tertiary structures). When the cells of the glioma are uniformly and evenly distributed, the structure is described as amorphous. The following secondary structures are described: perineural or neuronophagic growth, perivascular growth, superficial growth, perifascicular, intrafascicular and interfibrillary growth, and

elective growth in gray and white matter. The proper structures are classified as canalicular, papillary, fascicular and symplastic structures and ribbon and palisade formations. The secondary structures play a much more important role in the architecture of most gliomas than do the proper structures. In certain rare gliomas, having a strictly expansive type of growth (ependymomas), secondary structures do not develop. Secondary structures do not always develop in the infiltrating gliomas, sometimes because of the extremely rapid destruction of the preexisting tissue (medulloblastomas, certain glioblastomas), but certain other infiltrating tumors remain amorphous in spite of the perfect conservation of the preexisting tissue (typical astrocytomas). Three stages of structural evolution are encountered in the majority of gliomas. Various secondary structures are formed first, followed by an amorphous arrangement and finally by the various proper structures. There are marked differences in the structural development of gliomas. These variations must be considered as an expression of the fundamental biologic differences of the tumors themselves and must be taken into consideration in any future classification together with the localization, mode of extension and cellular appearance. Gliomatous structures differ in the same tumor depending on the area invaded (different secondary structures) and on the age of the tumor (different stages of the structural evolution). Stable structures in gliomas are rather rare. At present differences between gliomas in respect to structural evolution are unexplained. The etiologic explanation of the curious secondary structures is also unknown. A review of the literature suggests that carcinomas also show differences of structural development in the sense that either proper structures or secondary structures may appear to predominate. This suggests that there are broad and general laws which determine the morphologic evolution of all neoplasms.

FROM AUTHOR'S SUMMARY

ANTERIOR PITUITARIES OF OLD RATS. J. M. WOLFE, W. R. BRYAN and A. W. WRIGHT, *Am J Cancer* **34** 352, 1938

The anterior lobes of the pituitaries of old male and female rats over 18 months of age were studied and compared with those of younger animals. In those from the old rats it was found that the levels of eosinophils were significantly lower and those of the chromophobes were significantly higher than the levels of the corresponding cells in the younger animals. No important difference was found in the levels of the granular basophils in young and old rats, but nongranular basophils were significantly more abundant in the younger ones. In one strain of rats the incidence of benign mammary fibroadenoma in the old females was quite high, while in the other two strains it was much lower. In all three strains it was found that there was a tendency for the levels of the eosinophils to be lower in those rats bearing mammary tumors than in rats without tumors. There was no difference in the levels of the granular basophils in the two groups, but the nongranular basophils were more abundant in rats without mammary tumors. Chromophobes were generally more abundant in rats with mammary tumors. It was found that adenomatous changes of the anterior lobe of the pituitary occur frequently in old rats, particularly in females. Adenoma associated with marked hemorrhage and classified as hemorrhagic adenoma, chromophobe adenoma and an adenomatous nodule that was believed to be a forerunner of true adenoma are described. All quantitative data were handled by statistical methods.

FROM AUTHORS' SUMMARY

MODE OF ACTION OF METHYLCHOLANTHRENE. W. R. EARLE and C. VOEGTLIN, *Am J Cancer* **34** 373, 1938

The action of methylcholanthrene has been studied on rat and mouse fibroblasts. Under the usual cultural conditions methylcholanthrene severely retarded the growth and caused degeneration of the cultures, the effect being roughly proportional to the concentration of the reagent and the time of exposure. This action

was most accentuated with concentrations of 2 mg of crystalline methylcholanthrene per cubic centimeter but was also demonstrated with concentrations as low as 0.0002 mg per cubic centimeter. In a single extensive series of mouse fibroblasts studied in detail, in which wavelengths of light covering the absorption spectrum of methylcholanthrene down to 490 microns were eliminated, the same retardation of growth was observed as in the other series, but there was less cell degeneration. The retardation of growth cannot be attributed to the action of an impurity in methylcholanthrene. One series of cultures was studied with 1,2,5,6-dibenzanthracene. While conditions were not so carefully controlled as with the other series, the cultures showed a marked injurious action of this substance. In no instance in the experiments reported was any change observed in the cultures which could be considered as causing the normal fibroblasts to assume a morphologic character similar to that of the cells which were studied in growths arising as a consequence of the subcutaneous and intramuscular injection of methylcholanthrene into rats. In no instance was there noted any stimulative action of either the dibenzanthracene or the methylcholanthrene. The significance of these results is discussed in relation to those published by others.

FROM AUTHORS' SUMMARY

VITAMIN A AND TUMOR MITOCHONDRIA A. GOERNER and M. M. GOERNER,  
J. Biol. Chem. **123** 57, 1938

Vitamin A was not present in the mitochondria of tumor cells in either the Flexner-Jobling rat carcinoma or the R-39 sarcoma despite the fact that the hepatic mitochondria of both types of tumor-bearing animals contained a normal store of the vitamin as well as of total lipid. The total lipid content of the mitochondria of the tumor cell was fairly large in both experimental tumors but was higher in the Flexner-Jobling rat carcinoma than in the sarcoma. The results indicate that an increase in the vitamin A intake can have little effect on the growth processes of tumor cells.

R. J. LEBOWITZ

NEOPLASM OF MONOCYTES J. FURTH, J. Exper. Med. **69** 13, 1939

A transmissible neoplasm of mice characterized by malignant cells resembling histiocytes (monocytes) is described. The morphologic aspects of these cells and the microscopic appearance of the neoplasm are similar to those of the human neoplasm formed by histiocytes. The malignant histiocytes form tumor-like masses in the liver and spleen and infiltrate these and other tissues. When the disease is far advanced, they are present in small numbers in the blood of many mice. The malignant cells have scant phagocytic ability. The fixed cells of the host (endothelial cells and fibroblasts) have no significant part in the production of the lesions of the disease. Transmission is readily accomplished when material containing the malignant histiocytes is used for inoculation but fails in their absence. Attempts to demonstrate a cell-free transmitting agent have been unsuccessful.

FROM AUTHORS' SUMMARY

SOLUBLE ANTIGEN OF MYXOMA T. M. RIVKIN, S. M. WARD and J. E. SMADEN,  
J. Exper. Med. **69** 31, 1939

The soluble antigen of myxoma is a heat-labile protein which has an isoelectric point near  $pH$  4.5 and is precipitated from half-saturated solutions of ammonium sulfate. It can be partially purified by methods of differential precipitation based on variations in the  $pH$  and electrolyte concentration. In rabbits receiving the labile soluble substance of myxoma homologous precipitins develop, and their serum agglutinates elementary bodies of myxoma, provided the dermal pulp from which the bodies are obtained contains the soluble substance, neutralizing antibodies do not appear, however, and the animals are not resistant to infection with the virus of myxoma. Elementary bodies of myxoma appear to

have a heat-stable agglutinin which operates when brought in contact with serum from animals recovered from myxoma but which shows little action, if any, when in contact with anti-soluble-substance serum

FROM AUTHORS' SUMMARY

CARCINOGENIC EFFECT OF PAPILLOMA VIRUS J G KIDD and P ROUS, J Exper Med **68** 529, 1938

A considerable variety of tumors, both benign and malignant, result from the localization of the virus of rabbit papilloma in skin which has been prepared by repeated tarrings. They appear only in animals highly susceptible to the action of the virus, and are more likely to be engendered by highly pathogenic inoculums. No evidence has been found that differences in the potentialities of the virus entities are responsible for the diversity of the growths. This is referable to changes in the epidermal cells, and much more preliminary tarring is required to produce these changes than suffices to cause localization of the virus out of the blood stream, with a resulting papillomatosis of the ordinary sort. The character of the individual anomalous tumors depends in some degree on the extent of the preparatory changes in the cells, malignant growths being more frequent when the epidermis has been tarred for a relatively long period. All are focal or punctate in origin, and they exhibit their peculiar characters from the first, these characters not being due in any case to secondary alterations in ordinary papillomas. Tarring after the virus has localized in the epidermis does not significantly increase the number of growths. Their development is the outcome of the state of the cells at the time of virus infection. Tarring exerts important influences in addition to changing the cells in such a way that unusual tumors result from the action of the virus. The procedure is notably effective in determining localization of the virus out of the blood stream, it enables the virus to produce growths when otherwise it would not do so though present in the tarred skin, it stimulates the proliferation by which the tumors are engendered, it makes them disorderly and aggressive, and it hastens the anaplasia of such of them as are malignant. It has similar effects on the tar tumors, as will be demonstrated in a subsequent paper.

FROM AUTHORS' SUMMARY

FIBROMA VIRUS INFECTION IN TARRED RABBITS C G AHLSTROM and C H ANDREWES, J Path & Bact **47** 65, 1938

Rabbits which have been given intramuscular injections of tar respond in an abnormal way to the virus of infectious fibroma (OA strain). In tarred animals the regression of lesions produced by intradermal or subcutaneous inoculations of the virus is much delayed. In some tarred rabbits the subcutaneous tumors may even grow progressively and invasively until the animals die, coming to resemble true neoplasms both clinically and histologically. After intravenous inoculation of the virus into tarred rabbits, generalized fibromatosis commonly develops and may be fatal. In tarred rabbits antibodies to the virus develop, and the animals become immune to reinoculation in the same way as controls. A single dose of tar given on the same day as the virus produces the effects described, a series of inoculations is unnecessary. Benzpyrene and other carcinogenic hydrocarbons produce effects similar to those of tar, but it is not certain how far these effects are specific for carcinogenic substances. No effect of tar was detected in infections with inflammatory (IA) variant strain of the virus of fibroma nor with the virus of vaccinia.

FROM AUTHORS' SUMMARY

A SUPRASALLAR TUMOR IN A DOG E G WHITE J Path & Bact **47** 323, 1938

A suprasellar tumor in a dog, considered to be a craniopharyngioma of unusual structure, is described. The animal showed during life a syndrome resembling dystrophia adiposogenitalis in man.

FROM AUTHOR'S SUMMARY

MORPHOLOGY OF EPULIS L GERY and O NOEPPPEL, Bull Assoc franç p l'étude du cancer **27** 137, 1938

Gery and Noeppel define epulis as a sessile or a pedunculated outgrowth of the gum, originating from its soft tissue. They studied 240 such growths seen between 1919 and 1937 at the Institute of Pathology in Strasbourg, France, and found 230 of them to be hyperplastic tumors of the mesenchyma or, rarely, of the alveolodental ligament. All were benign, though some recurred locally. Histologically they could be divided into four not sharply separated groups: the inflammatory, the vascular or angiomatous, the fibrous and the giant cell group. Transitions among them were so frequent that a histologically homogeneous form was rare. The inflammatory epulis consisted of granulation tissue rich in blood vessels and in an exudate that sometimes was leukocytic but often plasmocellular. The vascular epulis showed excessive formation of blood vessels, which lacked the regularity and arrangement that are characteristic for granulation tissue. The fibrous epulis was made up predominantly of fibroblasts with varying amounts of intercellular fibers. Two types of cells determined the giant cell epulis: a small mononuclear cell resembling a fibroblast, but easily differentiated from it, and the well known multinucleated giant cell, similar to the bone marrow giant cell. The small cells, which could be shown to be connected with each other by means of protoplasmic outgrowths, are considered by Gery and Noeppel to be the fundamental unit of the growth. The hypotheses concerning the formation of the giant cells are discussed in detail. Their mesenchymal origin and lack of any relation to the bone marrow giant cells are emphasized, and the name "histioplaxe" is suggested for them. In all the forms of epulis deposits of collagenous masses were found, as well as ossification. The latter is explained as the function of the histiocyte in the sense of Maximow's polyblast. The deposition of calcium in the preformed osteoid tissue, as well as calcification without bone formation, is brought into relation with the observation that not infrequently decalcification of the alveolar process was recorded with transport of calcium, much as in callus formation. New-formed cartilage was observed very rarely. The authors emphasize the evidence that speaks against the origin of the epulis from bone. In their opinion, the epulis is formed by the reticulum in response to an unknown irritating factor. The histiocyte with its formative potentialities is fully capable of explaining the morphologic variations of the epulis and the frequent observation of phagocytosis.

I DAVIDSOHN

### Medicolegal Pathology

EPENDYMAL CYSTS CAUSING SUDDEN DEATH K BOHMFR, Deutsche Ztschr f d ges gerichtl Med **30** 59, 1938

Two deaths, supposedly resulting from accidents, were explained at autopsy by tumor occlusion of the aqueduct of Sylvius. In each instance the tumor was a cyst of ependymal origin, covered with connective tissue and lined by a layer of epithelium. The cyst content was rich in cholesterol and lipochrome. The first patient was a woman 46 years old who had complained of headaches for two months. She fell off a merry-go-round, struck her head on an iron rail and became unconscious for twelve hours. In the interval of two weeks between the time of the accident and her death she had headaches almost constantly. She died suddenly. The tumor was found in the third ventricle and was the size of a hazelnut. The accident two weeks before death was regarded as a result of temporary occlusion of the aqueduct by the tumor.

The second death occurred in a cyclist a day after he had been found unconscious beside a cycle path. After regaining consciousness, he thought he had been struck on the shoulder by another rider and that he had lost his balance and fallen. However, he had sustained no serious injury, and his bicycle was undamaged and had been set against the curb by him. The tumor in the aqueduct was only 2 mm in diameter. The lining of the third ventricle was roughened.

A reconstruction of the course of events seemed to justify the assumption that the man had exerted himself while riding uphill, disturbed the cerebrospinal fluid balance in the brain and caused the tumor, which it is assumed had theretofore floated freely, to clog the aqueduct. With the first evidences of cerebral compression from the lateral ventricles, the man dismounted, leaned his bicycle against the curbing and then sank to the ground in a stupor.

Bohmer cites cases reported by Beutler, Sjoval and Weissenburg to substantiate his interpretation of the cases reported. The theories of the cause of death in occlusion of the third ventricle are discussed briefly.

GEORGE RUKSTINAT

# Society Transactions

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## CHICAGO PATHOLOGICAL SOCIETY

KATHARINE M. HOWELL, *President*

*Regular Monthly Meeting, April 10, 1939*

EDWIN F. HIRSCH, *Secretary*

### RELATION OF CIRCULATING PRECIPITINS TO THE ARTHUS PHENOMENON PAUL R. CANNON and CHARLES E. MARSHALL

The uncertainty as to the role of circulating precipitins in the Arthus phenomenon has been due mainly to the use of inadequate methods for determining the precipitin content of serum. Dilutions of the antigen have been used in most studies, although this procedure is a titration of antigen rather than of antibody. We have studied the problem as follows: Collodion particles to which crystalline egg albumin had been adsorbed were added to serial dilutions of serum from rabbits immunized against crystalline egg albumin. By centrifugation and resuspension the antibody strength of such serums can be determined readily and correlated with the varying degrees of cutaneous reactivity to intradermal injections of crystalline egg albumin during immunization, after desensitization, after passive transfer and in other conditions. The results indicate that the Arthus phenomenon is associated invariably with the presence of specific precipitins in the serum of the animal and that the intensity of the inflammation parallels the precipitin content of the serum.

#### DISCUSSION

EDWIN F. HIRSCH: Do you have an explanation for the apparent lag in the appearance of the cutaneous sensitivity in reference to the appearance of the precipitin content of the blood?

I. DAVIDSOHN: This is a significant contribution since Opie's report.

PAUL R. CANNON: The precipitin content of the blood must be at a certain level—a concentration effective in dilutions of 1:240 or 1:480—before the skin reaction will occur. Then the skin reaction increases in proportion.

### OBLITERATION OF THE PLEURAL SPACE FOLLOWING PNEUMONECTOMY F. J. PHILLIPS and W. E. ADAMS

Cancer of the lung is being diagnosed earlier in the course of the disease because of increased diagnostic acumen and improved laboratory methods. The technical procedure of pneumonectomy is better as a result of the work of Graham, Churchill, Rienhoff and others. The anatomic and physiologic changes following pneumonectomy are not well understood. Following pneumonectomy the human pleural space becomes filled with a fibrous tissue shell by organization of the accumulated serofibrinous exudate. Mediastinal shift does not appear to be a dangerous hazard in these patients. The details of successful total pneumonectomy in a man aged 58 with squamous cell carcinoma of the left bronchus are presented. Nine months later he died from generalized peritonitis following rupture of the appendix. The fibrous tissue shell in the left side of the chest was 1 to 3 cm thick and contained a brown turbid fluid. Protocols of experiments in dogs on subtotal collapse and pneumonectomy demonstrate obliteration of the pleural space by hyperdistention of the remaining inflated lung tissue.

### REGIONAL FIBROCYSTIC DISEASE CARROLL O. ADAMS

Regional fibrocystic disease is a rare condition which is usually confused with other extensive cyst formations in bones. This report is the conclusion of a study

of 10 cases available for study at the University of Chicago Clinics. Two patients had involvement of an upper extremity only. These had a monomeric distribution, with involvement of the humerus and radius and, in 1 case, of the first metacarpal bone, without involvement of the ulna or the other bones of the hand. One patient had a small lesion in the humerus and massive lesions of the pelvis, femur, tibia and fibula on the same side. In another patient the ilium and femur on each side and the tibia on the left side were the loci. The other 6 patients had involvement in a lower extremity only.

Regional fibrocystic disease is probably congenital. This explains the varied extensiveness, the common segmental distribution and the usual onset of symptoms in childhood. The symptoms are mild aching pain following trauma, but pathologic fracture may be the first manifestation. These fractures usually heal, but delayed repair and malunion are common. Refracture is not rare, and nonunion occurred in 1 patient. The levels of the blood calcium and phosphorus are normal.

The roentgenograms usually showed the diaphysis expanded, especially in regions of previous fracture. The cortex was usually thin, and over the convex side of bowed long bones it was sometimes barely perceptible. In such regions fractures, complete and incomplete, may occur repeatedly. Occasionally, regenerative tissues developed in these weakened places. In the medullary region the normal trabecular pattern was replaced by irregular heavy trabeculae between which there might be decreased density and complete absence of trabeculae. In 2 patients with involvement of an upper extremity the humerus had this typical appearance, while the cortex of the radius was thin, and the entire medullary region was reduced in density and was without trabeculation. The epiphyses usually appeared normal.

The cortex of the bone was thin, and some of it could be sectioned with a knife. The marrow was replaced by yellow, gray or white fibrous tissue. Unlike the localized type, there were few cystic cavities, and the regions which appeared cystic in the roentgenograms were filled with fibrous tissue. In the regions which appeared devoid of trabeculae in the roentgenograms there were a few fine irregular trabeculae scattered throughout the fibrous tissue. Microscopically, the fibrous tissue was usually mature and dense. The cystic cavities present contained gelatinous material and were lined by compact fibrous tissue. The trabeculae had many irregular cement lines and occasionally some osteoid tissue.

Treatment was limited to the preservation of function, the prevention or correction of deformity and the curettement of regions with severe persistent pain.

#### BRONCHIAL OBSTRUCTION PRODUCED BY CARDIOVASCULAR ANOMALIES PAUL HOLINGER and A. H. ANDREWS

Tracheal or bronchial obstructions produced by cardiovascular anomalies are relatively rare. They have striking and frequent unusual clinical manifestations which generally are explained only by the postmortem examination. As in any bronchial obstruction, whether caused by an intrabronchial, an endobronchial or an extrabronchial lesion, that caused by a cardiovascular anomaly manifests itself by obstructive emphysema or atelectasis. Four representative examples were presented.

The first was in a 4½ month infant who had died of asphyxia from compression of the trachea. Postmortem examination revealed the aorta divided into two arches which surrounded and constricted the trachea where the branches passed backward and united posteriorly. The second patient, an infant of 5 months, had a partial bronchial obstruction with marked obstructive emphysema caused by a transversely placed distended right auricle which compressed the bronchus against the thoracic aorta. Numerous other cardiac anomalies, including atresia of the tricuspid valve, were present. In the third patient an atelectatic left lower pulmonary lobe was due to compression of the lung by a congenitally enlarged heart. In the fourth patient marked obstructive emphysema of the right lung had resulted because the aorta, by passing to the right of the trachea instead of to the left, had partially compressed the right main bronchus.



KATHARINE M HOWELL, *President**Regular Monthly Meeting, May 8, 1939*EDWIN F HIRSCH, *Secretary*

## STUDIES ON THE SEX RATIO (A) THE METABOLIC GRADIENT AS A CONDITIONING FACTOR, (B) THE SEX RATIO AND RESISTANCE WILLIAM F PETERSEN and MILDRED NORVAL

Apart from obvious differences, one basic biologic difference between the sexes resides in the fact that the male organism throughout its entire life span is geared to a higher oxidative rate, i e, it needs more energy in order to exist. The biologic inference seems obvious, for such a situation proves beneficial in providing greater opportunity for female survival and therefore for racial survival. If a large share of the energy expenditure is devoted to the maintenance of body temperature, the male may be expected to be less resistant to cold, first because of less fat tissue insulation, second, because of lesser opportunity to accumulate reserves than is afforded the female under similar conditions, and, third, because of greater demand on reserves during unusual stress or strain, such as cold, fatigue and illness.

An examination of the day by day sex ratio of persons dying in Detroit in the years 1934, 1935 and 1936 indicates that the ratio of males to females increases when masses of cold air pass over the region. On the other hand, during periods of mortality from undue heat (Detroit in June 1936 Chicago in July 1934) the trend of this ratio is relatively lower.

Despite the advantage of a generally lower basal metabolic rate for the female, it seems that adjustment to undue heat in general is more difficult for the female because of her greater peripheral insulation. This apparently finds expression, too, in the general experience of white persons domiciled in the tropics, where the female suffers more acutely and ages more rapidly than the male.

## DISSEMINATED GIANT CELL REACTION, POSSIBLY PRODROMAL MEASLES WALTER A STRYKER

In a 27 month old child dying of pneumonia multinucleated giant cells were found in the pulmonary alveoli, the bronchi, the sinuses and medullary cords of peribronchial lymph nodes, the lumens and walls of bronchial mucous glands, the interstitial connective tissue about lymph nodes and mucous glands, the spleen and the lymphatic tissue of the ileum. These cells were morphologically like those described as specific for the prodromal stage of measles. A history of possible contact with measles was obtained. Attempts to demonstrate inclusion bodies were inconclusive.

## DISCUSSION

I DAVIDSOHN The report is interesting, especially because of the widespread occurrence of the giant cells. The variety of giant cells is most amazing. May the nature of the disease be diagnosed on the basis of the microscopic structure of the tissues? I am inclined to think that the tissue structure probably indicates measles.

WALTER A STRYKER This case is not presented as a proved instance of giant cells in the prodromal stage of measles. The best evidence would be the demonstration of inclusion bodies in the giant cells in the prodromal stage as in the active stage of measles.

## DYSGERMINOMA OF THE TESTICLE J D KIRSHBAUM and MAURICE B JACOBS

Thirty malignant testicular tumors were selected for study in the department of pathology of the Cook County Hospital, 26 of the tumors were classified as dysgerminoma and 4 as mixed—i e, they contained dysgerminoma tissues

associated with cell cords and acini, the cell type, however, being uniform. The latter 4 tumors may be considered as representing an intermediate stage of a more highly specialized type of the dysgerminoma. Robert Meyer introduced the term "dysgerminoma," in 1930.

All of the tumors had a single type of cell, which, according to Robert Meyer, originates from the undifferentiated germ cell of the gonads. This conception explains the occurrence of similar tumors in ovaries. In size the tumors ranged from that of a walnut to that of a grapefruit. The tissues were moderately firm, encapsulated, homogeneous and gray-white. There were hemorrhages in 20 and necrosis in 25, of which 5 had caseation necrosis resembling tuberculosis, with tubercles but no Langhans giant cells. The tumor cells were large and polygonal, contained ample cytoplasm and had round or oval, deeply stained nuclei. Nucleoli were frequent, and cells with atypical mitosis were abundant. The arrangement of the cells was diffuse in 20 tumors, in the form of alveoli in 6 and after a papillary pattern in 4. Some had large irregular nuclei, often there occurred one that was multinucleated. The stroma frequently contained small lymphocytes, and narrow connective tissue trabeculae extended between alveolar masses of cells.

The youngest patient was 18 years of age, the oldest was 63. Fifty-seven per cent of the patients were in the third and fourth decades of life, while 58 per cent of the 12 patients examined post mortem were in the third decade. Twenty-seven patients were white and 3 were colored. The left and the right testicles were affected in equal numbers. Eleven of the patients gave a history of trauma preceding the onset of the tumor. Only 9 patients complained of pain on admission, while swelling was mentioned by 23. In 10 patients there was a loss of weight ranging between 20 and 35 pounds (9 and 16 Kg). Among the 18 patients treated surgically, the duration of symptoms varied from two to twelve months in 13 and from one to six years in the others. The clinical diagnosis of testicular tumor was correct for 18 patients, while for 12 an incorrect diagnosis, such as hydrocele or tuberculosis, was made.

In 12,000 consecutive necropsies from 1929 to 1939 only 15 malignant tumors of the testicle were observed—12 of them dysgerminoma, 2 chorioepithelioma and 1 malignant teratoma. The 12 patients with dysgerminoma had metastases, 10 extensive, especially in the lymph nodes, lungs and liver, and 2 had hematogenous dissemination into the lungs, spleen, brain and bones. Thrombosis of the inferior vena cava and iliac veins occurred in 5. One patient was noted in whom each testicle seemed to be the site of a primary tumor, the second tumor developing twelve years after the removal of the first. Another patient showed metastasis to the opposite testicle.

The term "dysgerminoma" may be used to designate the testicular or ovarian tumors which heretofore have been termed "seminoma," "spermatocytoma" or "embryonal cell carcinoma." The origin of a dysgerminoma is controversial. Ewing and Hinman consider the tumor to be teratoid, although only one type of cell may exist. The explanation offered is that by rapid growth the type cell supplants and suppresses the other elements. Chevassu and Bell used the terms "seminoma" and "spermatocytoma" and stated their belief that the cells are derived from the lining of the seminiferous tubules. Robert Meyer believed that the cells are not derived from the tubules but that they invade the tubules. Other testicular tumors described as malignant teratoma usually contain various structures of mesoblastic, hypoblastic and epiblastic origin.

#### DISCUSSION

WALTER SCHILLER. If the term "dysgerminoma" is accepted it should be used as Robert Meyer suggested. He found the same kind of tumors in ovaries unassociated with masculinization. He concluded that the growths arise from intersexual elements and produce no hormone. The prefix portion of the term implies origin in both sexes. Many of these growths appear with teratomas. In males the maximum incidence is between the ages of 30 and 40, in females between the ages of 10 and 15. The tumors in males are malignant, those in females, benign. In structure they are alike.

A B RAGINS Were determinations of gonadotropic substance made?

J D KIRSHBAUM Tests for such substance with 3 patients were negative

CAUSAL SIGNIFICANCE TO TRAUMATIC OSSIFICATION OF THE FIBROCARILAGE IN  
TENDON INSERTIONS EDWIN F HIRSCH and RUSSELL H MORGAN

The early stages of the lesion of traumatic ossification show large amounts of fibrocartilage or hyaline cartilage continuous with bone in varving degrees of differentiation Some portions seem to be ossifying cartilage, others have lamellar trabeculae containing residues of cartilage In the late stages the lesion has a high content of lamellar bone and only small traces of cartilage These conditions imply that bone has its origin in a cartilage matrix, i e, enchondral bone formation

Fibrocartilage is a normal constituent of the insertions of many tendons in which traumatic ossification occurs A reactive or reparative growth of these tissues initiated by trauma provides a simple explanation for the lesion of traumatic ossification

The complete report will be published in the *Archives of Surgery*

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NEW ENGLAND PATHOLOGICAL SOCIETY

CHARLES BRANCH, *President*

*Regular Meeting, April 20, 1939*

GRANVILLE A BENNETT, *Secretary*

A MICROSCOPIC STUDY OF THE SPHENO-OCCIPITAL SYNCHONDROSIS IN MONGOLISM,  
WITH A COMPARISON OF THIS GROWTH DISORDER WITH CRETINISM AND  
CHONDRODYSPLASIA CLEMENS E BENDA, Wrentham, Mass

Previous studies in mongolism by means of roentgenograms and anatomic measurements revealed a growth disorder which involves the proliferative growth of cartilaginous and membranous bones The purpose of the present investigation was to determine the nature of this disorder of growth by microscopic study The speno-occipital synchondrosis of 6 mongoloid patients, aged between 8 months and 30 years, revealed a lack of preparatory column formation of the cartilage and an absence of growth sequences The bone ridges were heavily ossified and the bony borders perforated by primary marrow cavities

The spenoethmoid synchondrosis was found to be open and without ossification in 3 cases Two vertebrae of mongoloid children, 2 days and 6 months of age, respectively, were examined That of the first showed an almost normal picture, while that of the 6 month old mongoloid child disclosed complete arrest of growth and formation of heavy transverse bone ridges

A comparison of the bone changes observed in chondrodysplasia, cretinism and mongolism reveals differences in these three conditions In chondrodysplasia there is a primary change in the cartilage with early absorption and ossification, whereas the membranous bones and sites of periosteal ossification are not involved In cretinism the transformation of cartilage into bone is affected, but the preparation of the cartilage is normal In mongolism there is a deficiency of growth in all epiphysal lines The cartilage fails to grow normally or to be absorbed This picture, together with lack of sinus formation in the bones of the skull, lack of diploe and absence of growth at the bone ends, is suggestive of congenital pituitary deficiency Evidence for such a conclusion has been accumulated by a study of 14 pituitaries of mongoloids, reported previously

TRAUMATIC EPITHELIAL CYSTS THOMAS G COGSWELL (by invitation) and  
RAYMOND H GOODALE, Worcester, Mass

Traumatic epithelial cysts, also called epidermoid or implantation cysts, occur more frequently than is usually recognized. Comparatively little has been written about them, especially in American literature, and they are barely mentioned in the textbooks of this country. The traumatic origin has apparently been accepted universally, but the epithelial source is still subject to controversy. Several theories have been advanced, but that which sees the development of such cysts from sudoriferous glands and their ducts seems to be the most satisfactory.

The cysts appear as small round or oval swellings, which are usually nontender and somewhat freely movable. The common sites are the palms of the hands, the flexor surfaces of fingers and thumbs, the soles and exposed areas of the face. The greater incidence is in males. Microscopically, the cyst wall strongly resembles the epidermis, and the contents are cellular debris and dense keratin. Traumatic epithelial cysts are most easily confused with sebaceous cysts. It should be noted, however, that they appear on areas devoid of sebaceous glands or hair follicles and also that they exhibit differences observable in microscopic examination.

These cysts rarely become malignant and seldom become infected. An epithelial cyst in the pulp of a thumb or a finger may erode into the phalanx. The treatment is excision. There is a tendency to recur if removal is not completely accomplished.

#### DISCUSSION

B E CLARK, Providence, R I. I fear all are prone to pass over commonplace and benign lesions too lightly, with a feeling that there is nothing more to be learned about them. Probably many are accustomed to group all these epithelial-lined cysts together as epidermal cysts or something similarly designated. One may have in mind the possibility that some are of traumatic origin, that others result from obstruction to glands and that others come from embryonal rests, but one makes little or no attempt to find morphologic differences that may indicate the origin. Then some one with a little more curiosity makes a careful study, such as Dr Cogswell has just presented, and teaches one that there are morphologic differences which others have not noted or have failed to interpret.

F P MCCARTHY, Boston. At a large dermatologic clinic (50,000 visits and 8,000 new cases) my associates and I see only one or two of these epidermal cysts each year. I assume that most of the patients with such lesions go to the surgeon rather than to the dermatologist. Many are assumed to be sebaceous cysts and are treated accordingly, without histologic examination. Cysts of the type described do not tend to become infected as often as the sebaceous cysts. They are smaller, deeper, firmer, and often show a hyperkeratotic reaction of the skin covering the lesion.

LEO ALEXANDER, Boston. My colleagues and I have seen occasional instances of epidermoid tumor located intracranially. In the case of a woman, a report of which has since been published by Drs Munro and Wagner, the tumor was found extradurally in the right frontal region. This woman gave a history of having incurred a skull fracture in early childhood, involving that region, and it is reasonable to assume that epidermal material may have been implanted in the epidural tissue at that time. In other cases the traumatic origin was less obvious—for instance, in those cases in which such a tumor was seen in the cerebellum.

Recently in an apparently similar case in which Dr Munro operated two discrete, partly calcified nodules were found within the pia at the convexity of the brain posterior to the central region. However, in this case the differential diagnosis from small meningiomas with partial fatty degeneration has not yet been ascertained. I should like to ask Dr Cogswell whether he has observed calcification in any tumor in his cases.

B E CLARK, Providence, R I Dr Cogswell mentions the foreign body reaction which occurs about some of these cysts This is a common observation, and I have interpreted it as being due to a break in the epithelial lining with escape of the sebaceous content into the tissues, this material then stimulating the foreign body reaction I wish to ask whether Dr Cogswell feels that this is characteristic of cysts which are traumatic in origin Or may it not occur with so-called wens or cysts of other origin?

J E PORTER, Fall River, Mass What is the interval from the time of the trauma until the cyst is recognized?

ROBERT FIENBERG, Westfield, Mass At one time I was interested in the contents of epidermal cysts and extracted the material with fat solvents I found it to be predominantly keratin This finding probably explains the giant cell reaction in the tissues about the cyst, since the keratin might penetrate the corium through a break in the epithelial lining Certainly, when in heavily irradiated keratinized epidermoid carcinomas the cancer cells are destroyed, leaving necrotic masses of keratin, foreign body giant cell reaction is often noted about the keratin

Assuming that these epidermal cysts arise from sweat glands, it is interesting that Dr Cogswell was able to differentiate the epithelium found in the epidermal cysts from that found in the sebaceous cysts Since both arise from cutaneous appendages, the assumption would be that the metaplasia which occurs in these cysts would give rise to the same type of squamous epithelium in each kind of cyst Certainly, in most cases of squamous metaplasia one does not usually distinguish types of squamous epithelium

RAYMOND GOODALE, Worcester, Mass We have seen no malignant tumors in our series These cysts are prone to recur The recurrences may be due to a failure of the surgeon to remove tiny diverticula of the cysts Frequently these cysts are more difficult to excise than sebaceous cysts Complete excision is essential for a cure

THOMAS G COGSWELL, Worcester, Mass Unfortunately we have seldom had complete clinical information However, in a few cases we have observed hyperkeratosis of the overlying skin No intracranial lesions have been seen in our series of cases Reports of epidermoid cyst of the brain were found in the literature The so-called cholesteatoma involving the middle ear region is regarded as a similar lesion We have confined ourselves to a study of the cysts of the skin It is logical to believe that giant cells may be present as a reaction to the dense masses of keratin if there is a break in the wall which would allow keratin and other material to be passed out into the surrounding tissue It is interesting to note that no such phenomenon was found in any of the sebaceous cysts

Replying to the questions concerning trauma, it should be pointed out that the injury may be so slight as to pass unnoticed It may be said that a deep penetrating trauma is definitely not necessary for the development of such a cyst The cause may be constant irritation or repeated trauma, and no time element can be determined In certain instances there may be a single traumatic injury which is well remembered by the patient Two or three months are usually required for the development of the cyst, but many months or perhaps years may pass before clinical symptoms are manifested Certain factors, especially the site of the lesion, will have a bearing on the time element

#### LESIONS OF THE NERVOUS SYSTEM IN HYPERINSULINISM H M ZIMMERMAN, New Haven, Conn

The experimental animal employed in this study was the cat From 10 to 20 units of insulin per kilogram of body weight was injected, and by frequent analysis of the blood for sugar it was determined that the period of hypoglycemia extended for about ten to fifteen hours About 30 per cent of these animals recovered completely, and about 40 per cent did not survive the procedure The remaining

30 per cent survived but showed a condition approximating decerebrate rigidity. These animals were semiconscious, insensitive to painful stimuli, ataxic and spastic. Their blood sugar levels at this time were within normal limits.

Chemical analysis of the brains by Dr Herman Yannet disclosed a marked loss of cell water and a consequent increase in extracellular water, but the total water of the brain was not changed. There was an even greater loss of cellular potassium, with a shift of sodium into the cell. These changes were interpreted as indicating a loss of selective permeability of the cellular membrane, probably due to cellular disintegration.

A close correlation was found between the severity of these chemical changes and the anatomic lesions in the brain. The latter consisted of widespread cortical necrobiosis affecting the ganglion cells, which at first showed liquefaction and pericellular encrustation and later disappeared. The same type of change was found in the cornu ammonis formations, the basal ganglions and the cerebellum. Under certain conditions, especially when the duration of the hypoglycemia was short, these changes were apparently reversible, and the animal recovered both functionally and anatomically. Changes which are identical with these have been observed in both rabbits and men under conditions of hyperinsulinism with resulting hypoglycemia.

The genesis of these lesions is probably explained on the basis of a lack of dextrose in the brain resulting in "cellular starvation", since the brain is dependent for its nutrition in great part on the dextrose brought to it by the blood. Cerebral anoxemia produced by vascular occlusion produces focal lesions rather than the diffuse involvement seen in hypoglycemia. The anoxemia produced by such convulsants as thujone, camphor and metrazol, even when severe, never leads to such widespread destruction of ganglion cells, even though the type of cell injury is the same.

#### DISCUSSION

QUESTION Were hemorrhages found in the brains of these animals?

VALY MENKIN, Boston In regard to the mechanism of cytolysis, have you had the opportunity to study the local changes in  $p_H$ ? I have had no experience with the tissues which you are studying, but at least in exudates polymorphonuclear leukocytes are definitely injured by a fall in  $p_H$ . Incidentally, my coworkers and I also have been able to show that accompanying an inflammatory reaction there is an increase in the potassium of the exudate as compared with that of the serum. This is probably associated with injury to the cells. In our studies the local acidosis was referable to disturbances in the intermediary carbohydrate metabolism. This favors development of true local acidosis from lactic acid at the site of the injury. I wonder whether there is any possibility that a similar picture might present itself in Dr Zimmerman's experiments on hypoglycemia. I should therefore be interested to know whether he has had the opportunity to study glycolytic changes at the site of ganglion cell degeneration.

LEO ALEXANDER, Boston Dr Zimmerman has been the first to demonstrate clearly that changes similar to those which one observes following occlusion of vessels and which one therefore tends to accept as due simply to anoxia may be brought about by lack of a substance other than oxygen in the circulation, namely, dextrose. Although the general distribution of the change is not similar to that of the change brought on by simple anoxia, the cytologic change is rather similar to the so-called ischemic change of Spielmeyer, particularly, the analogy of the change in Ammon's horn is most striking. Dr Zimmerman's paper demonstrates most ingeniously that there are at least two components in the so-called ischemic change, that of anoxia and that of lack of dextrose.

We have had the opportunity in our laboratory to study a case of death following insulin shock in man. Death occurred in the acute stage of shock. An enormous degree of cerebral vasodilatation was observed in both upper parietal regions symmetrically. I should like to ask Dr Zimmerman whether in his cases the vascular system showed gross morphologic abnormalities.

The first one to suggest that the apparent improvement in schizophrenia following a series of insulin shocks may be due to destruction of cerebral tissue, which allows adjustment on a lower integrative level, was Dr Stanley Cobb, of Boston. I do not think that he intended necessarily to regard this fact as a strict contraindication to the treatment, but rather as a fact which might explain the mechanism involved in the apparent improvement. Dr Zimmerman's paper and clinical experience have since confirmed Dr Cobb's point of view. The question of whether the therapeutic use of insulin shock which destroys part of the brain is promoting the social adjustment of the schizophrenic patient will have to be decided from the study of a great amount of clinical material.

T DENNIE PRATT, Boston (by invitation). Recently, while working under the direction of Dr Oliver Cope at the Massachusetts General Hospital, I carried out an experiment with 2 adult male dogs in which the period of hypoglycemia induced by insulin was prolonged for forty-eight hours, except for one or two short intervals the level of the blood sugar was kept well below 40 mg per hundred cubic centimeters, with most of the readings being around 30 mg.

Profound objective neurologic changes appeared. Extreme prostration, together with spastic paralysis of the extremities, and complete failure to react to varying types of external stimuli were observed on the second day of the experiment. This stage was followed by a gradual return toward the normal, during which the dogs exhibited fairly typical examples of the rage phenomenon described by Cannon as being characteristic of the decorticate animal. In the succeeding two to three weeks the dogs slowly but definitely returned to their original normal state. No histologic studies have as yet been made.

In view of these objective findings I should like to ask Dr Zimmerman if he thinks it likely that these dogs could have had at one time the histologic changes which occurred in his cats, since the dogs now appear to have recovered completely. If so, one might conclude that either (1) the histologic changes are temporary or (2) the brain of the adult dog has a certain supplementary reserve by means of which the unaffected cells are enabled to take over the functions of the cells permanently damaged by the extreme hypoglycemia.

H M ZIMMERMAN, New Haven, Conn. Only two of the animals had hemorrhages, insignificant ones, and none of them showed any striking vasodilatation. Determinations of  $p_H$  on some of the more severely involved animals showed changes to the acid side. These changes, however, may have been the result of the extensive cellular destruction rather than the factor responsible for them. Animals which were hypoglycemic for less than four hours usually showed normal responses almost immediately thereafter. Those that were hypoglycemic for much more than four hours remained out of touch with their environment until put to death.

Replying to the question concerning areas of involvement — there appears to be no predilection for the parietal lobe. If anything, it is the occipital lobe that suffers the most.

One question pertained to clinical evidence indicative of cerebral injury in patients treated with insulin shock. Recently, I have been informed by Dr Fritz Kant, who has had experience with a large number of such patients, that following repeated shocks even those patients who respond favorably show evidence of this injury. Psychologic tests indicate that they are brought to a lower intellectual plane by this form of therapy.

ARTERIOSCLEROSIS M C WINTERITZ, New Haven, Conn.

The extensive vascular supply to the wall of artery and vein necessitates consideration of the role this may play in the disease of the vessel wall. Hemorrhages are frequently encountered in all of the coats, particularly in the intima. The fate of this hemorrhage can be traced through the breakdown products of the erythrocytes. These stain for iron and for fat in various stages of their

disintegration This is so for some cells still identifiable in the atheroma and in vessels at its periphery both when they are free and when they are within the bodies of large macrophages All of the iron within the macrophages probably is derived from the red blood cells The fat may well arise from other sources That much of the fat within the macrophages may be derived from red blood cells has been demonstrated by the injection of various irritants into the peritoneal cavities of rabbits When red blood cells occur in the exudate, the macrophages are laden with fat

Intimate association between artery and neighboring vein has been established by injection and clearing methods When the vein wall or the adventitia of the vein is inoculated with various micro-organisms, lesions of both the vein and the artery follow These are dependent largely on the virulence of the organisms In the vein, acute phlebitis and also thrombosis may result, and later, if the animal survives, either organization of the thrombus or a fibrous intimal plaque follows The arterial lesion is associated with exudation, more marked in the adventitia and in the intima than in the media Intimal proliferation follows rapidly Thrombosis of the artery may occur, or if the process does not extend to the lumen of the artery, it may result in progressive thickening of the intima Vascular lesions have been found to occur in another and quite different condition Hypertension and azotemia, produced by the Goldblatt method, are associated with lesions of the media of the larger arteries and veins Edema of the media and extravasation of blood in this coat of the vessel, extending into the intima but without loss of continuity of the intimal surface, occurs frequently in these larger vessels This is the analogue of the arteriolar lesions that occur under these circumstances, as described by Goldblatt and his associates

#### DISCUSSION

H E MACMAHON, Boston I should like to ask Dr Winternitz what procedure he follows in obtaining these very striking pictures showing vascularization with hemorrhage throughout the wall of the aorta, also, what part of the aorta most frequently shows these lesions, and at what age they most commonly occur

In a study of the vascular lesions of lungs in cases of long-standing pulmonary tuberculosis one may find within the vascular walls every change that has been shown here In a comparatively limited study of the aorta in younger patients showing marked hypertension I have been unable to find such striking pictures as have been shown by Dr Winternitz This, I feel, is largely due to the technic, for the clearing process was not used in the selection of sites for study

In a recent paper Neumann and co-workers (*Nuchows Arch f path Anat* **303** 1, 1938) pointed out that the first portion of the aorta, extending up to the arch, is supplied by branches from the coronary arteries I should like to ask Dr Winternitz if he has had an opportunity to study this particular vascular tree

CLEMENS E BENDA, Wrentham, Mass I should like to ask Dr Winternitz if any type of infectious process produces the lesions in blood vessels which he has shown to us, also how widespread they are

B E CLARK, Providence, R I I should like to ask whether or not vascular lesions of this type may serve in explaining idiopathic cystic medial necrosis

M C WINTERNITZ, New Haven, Conn Clearing of the vessel wall is of great importance in demonstrating hemorrhage Extensive sectioning of the vessel wall with a sharp instrument is also of great aid It should be emphasized that in such manipulations damage to the intima by such procedures as sponging and washing should be avoided The iron stain as well as stains for fat are of value in demonstrating the participation of red blood cells in the process of disease of the vessel wall A possible relationship between these medial hemorrhages and idiopathic medial necrosis may be suggested



## Book Reviews

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**Problems of Ageing Biological and Medical Aspects** Edited by Edmund Vincent Cowdry, A B, Ph D, Professor of Cytology, Department of Anatomy, Washington University Cloth Pp 749, with 121 illustrations Price \$10. Baltimore Williams & Wilkins Company, 1939

The book is a product of the interest of the Josiah Macy Jr Foundation in chronic degenerative conditions and impairments with special reference to aging. As stated in the editor's preface, the book is a logical sequence of the survey of the problem of arteriosclerosis issued under the same auspices in 1933, in which is summarized the knowledge of degenerative changes and aging of blood vessels (Cowdry, E V, editor *Arteriosclerosis A Survey of the Problem*, New York, The Macmillan Company, 1933). In his foreword, Lawrence K Falk, of the Foundation, describes the general scope and purpose of the book as conceived and carried out by the editor, namely, to advance the "larger conception of health-care to which the Foundation" is dedicated. The book deals not only with the involutions of uncomplicated aging—"the gradual running down of the hereditary 'time-clock'"—but inevitably also with changes in the aged due to degenerations and impairments other than those inherent in physiologic aging. This distinction and the interrelations of the two sets of processes naturally give rise to much discussion. In his introduction, John Dewey emphasizes the relations between aging and the social life. He says "Science and philosophy meet on common ground in their joint interest in discovering the processes of normal growth and in the institution of conditions which will favor and support ever continued growth." There are 25 chapters in which phases and problems of aging are discussed by well known investigators in various fields. A list of the chapter headings and authors will show best the ground work covered.

Ageing of Plants—William Crocker

Senescence and Death in Protozoa and Invertebrates—H S Jennings

Ageing of Insects—L O Howard

Ageing of Vertebrates—T Wingate Todd

Human Cultural Levels—Clark Wissler

Longevity in Retrospect and Prospect—Louis I Dublin

Cardiovascular System and Blood—A E Cohn and Karl Landsteiner

Lymphatic Tissue—E B Krumbhaar

Digestive System—A C Ivy

Urinary System—Jean R Oliver

Skeleton, Locomotor System and Teeth—T Wingate Todd

Ageing of the Skin—F D Weidman

The Thyroids, Pituitary, Adrenals, Thymus and Islands of Langerhans—  
A J Carlson

Female Reproductive System—Edgar Allen

Male Reproductive System—Earl T Engle

Changes in Personality and Psychosexual Phenomena with Age—G V.  
Hamilton

Ageing of the Nervous System—Macdonald Critchley

The Eye—Jonas S Friedenwald

The Ear—Stacy R Guild

Psychological Changes—Walter R Miles

Chemical Aspects of Ageing—C M McCay

Ageing of Homeostatic Mechanisms—Walter B Cannon

Ageing of Tissue Fluids—E V Cowdry

Ageing Processes Considered in Relation to Tissue Susceptibility and Resistance—Wm DeB MacNider

Ageing from the Point of View of the Clinician—Lewellys F Barker

Each chapter concludes with a summary and a bibliography. For the editor and the contributors there can be nothing but praise. The book gives an excellent and unique recapitulation of the knowledge and understanding of the processes and manifestations of aging. It clarifies the present state, trends, and need of investigation in the field of aging.

**William B. Wherry, Bacteriologist** Martin Fischer Pp 293 Springfield, Ill., and Baltimore, Md., Charles C. Thomas, Publisher, 1938

This book is written for those who knew and loved the rare spirit that in the flesh was called William B. Wherry, it is not primarily for those whose chief interest would be to learn of the bacteriologist and scientist. The sources for material have been a vast accumulation of letters, 82 articles written in whole or in part by Wherry and an intimate friendship between Wherry and the author that began in medical school and lasted some thirty-five years, until the former's death in 1936. Skilfully drawing now on one source and now on another, Fischer tells of Wherry's boyhood interest in nature, his struggles with poverty, his strong home attachments and the gradual growth of more liberal views as to life than those held by his missionary parents. The student days at Rush Medical College are described, and the early contact with Hektoen, whose wise advice was for years an encouraging and much appreciated stimulus to productive research. One follows Wherry to Manila, California, Montana and many other places until finally, after wandering Odysseus-like in many lands, he reaches what was to be his final home in Cincinnati, where, as one of a congenial and understanding group, he continued his investigations and became an inspiring teacher, a leader in medical circles, an honored citizen and a beloved colleague and friend.

The author's task has been a labor of love. No incident is too trivial to record if it shows the dutiful son, the loving husband or the loyal friend. No excerpt from his writings is insignificant if it adds to the credit account of Wherry's investigations and discoveries in the field of bacteriology. Leprosy, plague, tularemia, amebiasis, improved culture mediums and methods of staining, more accurate technique for animal experimentation, original ideas as to the causation of disease, problems of immunity, the growth of a broad philosophic outlook on the nature of man and his diseases—all this is told with the charm of the easy style for which Martin Fischer has become well known. Occasionally there is pungent irony to an epigram. But the "Fischerisms" are not viciously barbed. The author is evidently trying to exercise restraint lest he be viewed as a too ardent advocate or an indiscriminating hero worshiper. Some critics may, in fact, think he is at times extravagant. He tacitly endorses the statement of another that when Wherry died "the last and the greatest of the bacteriologists of our period" had gone. Such critics might—naming only one—cite Theobald Smith as in all respects a greater bacteriologist. Theobald Smith, by the way, was one with whom Wherry came in contact and whose helpful influence he freely acknowledged. The number of other friends was great. Hektoen has already been mentioned. Others were Fischer, Ricketts, E. O. Jordon, Gideon Wells, Rosenow, Musgrave, Strong, McDill, as well as many unnamed college and hospital colleagues and students.

While, as the title of the biography declares, Fischer is considering Wherry chiefly as a bacteriologist, it is clear that he regards him—and readers must admit that he makes out his case—as worthy of remembrance not alone because he achieved renown in his chosen field of science but as well because he stimulated to sane thinking, advocated a broad philosophic view of life and by example and precept taught the value of mutually helpful friendship. It is a pleasure to add that the volume has been brought out in a form that is a credit to the publishers as well as to the author.

## Books Received

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THE BIOLOGY OF BACTERIA AN INTRODUCTION TO GENERAL MICROBIOLOGY  
Arthur T. Henrici, M.D., Professor of Bacteriology, University of Minnesota  
Second edition Cloth Pp 494, with 112 illustrations Price \$3.60 Boston D. C.  
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## CARCINOMA CELLS IN THORACIC AND IN ABDOMINAL FLUIDS

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For over fifty years there have appeared reports on the search for and the recognition of carcinoma cells in fluids aspirated from the thoracic or the peritoneal cavity. However, few clinicians think of requesting such examinations, because many of the pathologists associated with them consider it improbable that such a search will consistently yield conclusive diagnostic results. The favorable opinion of such examinations presented here is based on the findings in 100 cases in which carcinoma was being considered in the differential diagnosis and in which such an examination was made by a uniform technic.

Quincke<sup>1</sup> in 1875 was probably the first to see, describe and report carcinoma cells in smears from pleural and abdominal fluids. Since then there has appeared a long series of reports<sup>2</sup> of such findings in stained smears, always with a changing and presumably improving technic. Many of these reports, although based on observations of smears from but one or a few fluids, present elaborate discussions and numerous illustrations of the distinguishing minute peculiarities of the individual carcinoma cell. In the comment on such smears, emphasis is placed on such characteristics as the presence of mitotic figures, of clumps of cells

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1 Quincke, H. *Deutsches Arch f klin Med* **16** 121, 1875, **30** 580, 1882

2 Neelsen, F. *Deutsches Arch f klin Med* **31** 375, 1882. Dock, G. *Am J M Sc* **113** 655, 1897. Warthin, A. S. *M News* **71** 489, 1897. Gulland, G. L. *Scotch M & S J* **10** 490, 1902. Labbe, Delille, A., and Agumet. *Bull et mem Soc anat de Paris* **4** 507, 1902. Gwyn, N. B. *Am J M Sc* **127** 592, 1904. Miller, J. L. *Am Med* **8** 835, 1904. Turton, E. *Practitioner* **74** 497, 1905. Ross, E. A. *Tr Path Soc London* **57** 361, 1905-06. Vickery, H. F., and Richardson, O. *Boston M & S J* **157** 824, 1907. Facchini, G. *Policlinico (sez prat)* **15** 977, 1908. Marini, G., and Fiorini, M. *Riforma med* **24** 1, 1908. Widal, F., and Abram, P. *Bull et mem Soc med d hop de Paris* **25** 335, 1908. Warren, L. F. *Arch Int Med* **8** 648, 1911. Judd, C. C. W. *Am J M Sc* **153** 717, 1917. Femblatt, H. M. *M J & Rec* **121** 609, 1925. Quensel, W. *Acta med Scandinav* **68** 427 and 458, 1928. Karp, H. *Ztsch f Krebsforsch* **36** 579, 1932. McDonald, J. R., and Broders, A. C. *Arch Path* **27** 53, 1939.

or of vacuolated cells. Variations in the size and shape of the nuclei are considered important. Some lay special emphasis on the nucleus-cytoplasm or the nucleus-nucleolus ratio.

At first my co-workers and I also utilized direct smears and tried various staining methods, without much success. In some of these smears we found a few cells strongly suggesting carcinoma cells. In the paraffin sections made from the sedimented cells from these same fluids there were innumerable regions easily recognizable as neoplastic. Most pathologists still agree with Borst<sup>3</sup> that individual neoplastic cells, even malignant ones, have no absolute specific morphologic characteristics such as (1) degree of dedifferentiation, (2) irregularities of such cytoplasmic details as granules, mitochondria or golgi network, or (3) nuclear changes, such as hyperchromatism or hypochromatism or abnormality of centrosomes or of nucleoli. Cytologic identification of the individual carcinoma cell is for the present not uniformly possible.

Nevertheless, with all these limitations in mind, it is usually not difficult to identify as neoplastic a comparatively small group of epithelial cells in tissue sections. This recognition is based as often on the relation of these epithelial cells to one another and to the surrounding structures as on the minute characteristics of the cells themselves. It is very difficult to preserve this structural picture in smears, hence the decision there must rest almost entirely on cytologic details. The method of centrifuging these fluids and embedding and sectioning the sedimented cells permits the study of whatever histoid characteristics are present and for this reason alone it is a more reliable technic.

Scattered instances are recorded<sup>4</sup> in which a small bit of tissue was found floating in the aspirated fluid. This bit of tissue, separately embedded and sectioned, served best for the diagnosis. However, such accidental findings cannot be relied on for a diagnostic method, and as early as 1901 Josefson<sup>5</sup> advocated centrifuging these fluids and sectioning the sedimented cells. Even before this, in 1895, Bahrenberg<sup>6</sup> published a similar technic in the little read *Cleveland Medical Gazette*. In 1917 Mandlebaum,<sup>7</sup> of the Mount Sinai Hospital, New York,

3 Borst, M. Die Lehre von den Geschwulsten, Wiesbaden, J. F. Bergmann, 1902.

4 Prentiss, D. W. Tr. A. Am. Physicians **8** 191, 1893. Rieder, H. Deutsches Arch. f. klin. Med. **64** 544, 1895. Steiner, W. R. Bull. Johns Hopkins Hosp. **12** 310, 1901. Steele, J. D., and Girvin, H., Jr. Proc. Path. Soc. Philadelphia **4** 164, 1901. Nattau-Larrier, M. L. Compt. rend. Soc. de biol. **58** 709, 1905. Levesque, J. Medecine **5** 958, 1924. Bock, E. Klin. Wchnschr. **4** 651, 1925.

5 Josefson, A. Hygiea **63** 435, 1901, Ztschr. f. klin. Med. **82** 331, 1916. Acta med. Scandinav. **53** 770, 1921.

6 Bahrenberg, L. H. P. Cleveland M. Gaz. **11** 274, 1895.

7 Mandlebaum, F. S. J. Lab. & Clin. Med. **2** 580, 1917.

reported using this method, but it was not until 1928 that Zemansky<sup>8</sup> published his report of a series of 113 such examinations made in that institution. Meanwhile, Ellis,<sup>9</sup> of the Memorial Hospital, New York, in 1922 emphasized the value of a similar technic, and Foot<sup>10</sup> in 1937 again reviewed the material from that hospital. Also, Seecof and Boetsch<sup>11</sup> in 1924 reported on 93 cases studied by a similar method at the Montefiore Hospital, New York.

#### TECHNIC

The technic followed is extremely simple. The fluid is centrifuged at high speed until the supernatant liquid is clear. This is most conveniently accomplished by centrifuging repeatedly in the same 50 cc. round bottom centrifuge tube, pouring off the cleared fluid and replacing it by fresh fluid to be centrifuged. Sufficient sediment is thus finally obtained as one mass at the bottom of the centrifuge tube. Even if the fluid has not been collected aseptically, its preservation in the ice box for twenty-four or thirty-six hours before centrifuging permits little effect on the neoplastic cells. Also, centrifuging for a considerable period at the highest speed obtainable does not damage the cells.

The sedimented mass obtained is then prepared for fixation. It is first transferred to a piece of filter paper as a small compact pile. This transfer is made either with a small spatula, the point of a scalpel or a small platinum loop, depending on the amount and consistency of the sediment. The sediment obtained is seldom insufficient for such transfer, especially the sediment from fluids containing neoplastic cells. Sometimes a fibrin clot forms in the fluid, and then there is obtained after centrifuging a voluminous jelly-like precipitate, which comes out of the tube en masse. In this clot are enmeshed all the cells of the fluid. Before fixing, some of the fluid that is also enmeshed in the clot is extracted by gently rolling the clot around on dry filter paper. The sediment or clot is fixed in at least 10 volumes of a neutral 1:10 dilution of solution of formaldehyde U. S. P. for eighteen to twenty-four hours, embedded in paraffin, sectioned at 8 to 10 microns and stained with hematoxylin and eosin. This method differs from the original method of Josefson,<sup>5</sup> who added alcohol to the fluid before centrifuging and thus obtained a more voluminous precipitate of coagulated protein. Ellis<sup>9</sup> mixed solution of formaldehyde instead of alcohol with the fluid. Both these procedures yield a flaky, nonadhesive precipitate, which is difficult to handle and section. It has not been found necessary or desirable to fix the precipitate directly in the centrifuge tube as practiced by Mandlebaum<sup>7</sup> and by Zemansky.<sup>8</sup>

#### PATHOLOGIC PICTURE

Graham,<sup>12</sup> who used a comparable technic on 50 such fluids, has presented the most recent extensive discussion of the appearance of carcinoma cells in sections of the sediment of effusions. Although we have encountered all the pictures he so well described, we have never felt it safe to base a diagnosis on the appearance of individual cells. In

8 Zemansky, A. P., Jr. *Am. J. M. Sc.* **175** 489, 1928.

9 Ellis, E. B. *Bull. Internat. A. M. Museums* **8** 126, 1922.

10 Foot, N. C. *Am. J. Path.* **13** 1, 1937.

11 Seecof, D. P., and Boetsch, N. *Proc. New York Path. Soc.* **24** 2, 1924.

12 Graham, G. S. *Am. J. Path.* **9** 701, 1933.

every instance we have made the diagnosis of carcinoma only after finding a group of cells whose arrangement left no doubt as to their epithelial nature. The individual cells comprising the group were incontrovertibly separated by definite cell walls. The cells had a definite polygonal shape, often cuboid to high columnar. The group of cells having these characteristics may have been small in number, sometimes comprising only 4 to 6 cells. These few cells, however, were definitely arranged in the form of an acinus or a part of an acinus. In such an arrangement the nuclei of the cells exhibited definite polarity, all being located in the same position relative to the lumen or to the periphery of the acinus. These criteria for the recognition of carcinoma cells can be amplified best by discussing a few illustrative cases with their accompanying photomicrographs.

**CASE 1—*Carcinoma of the Ovary*** (fig. 1)—A 46 year old woman had swelling of the abdomen, dyspnea, anorexia and some loss of weight for three weeks. Examination showed only marked ascites and edema of the sacrum and ankles. The diagnosis was held in abeyance until after a paracentesis had been performed. Then a mass in the pelvis could be felt, and carcinoma of the ovary was suspected.

**Sections**—The initial few routine sections from the sedimented cells of the abdominal fluid were inconclusive in that, though a few suspicious clumps of cells were encountered, there was nothing of absolute diagnostic value. In figure 1 (1) are two clumps of cells, obviously different from the isolated cells sprinkled about them and resembling epithelial cells to the point of arousing a suspicion of carcinoma. The upper, larger clump, *A*, is the least epithelial-like of the two, but careful focusing on these cells showed definite cell walls between them in many places. A sharply defined, limiting cell wall between two contiguous cells is one of the most useful criteria in recognizing epithelial cells. The lower, smaller group, *B*, composed of only 4 cells, is more definitely epithelial, for in addition to sharply defined cell walls, seen on focusing, the cells show some polarity. Aiming to examine more closely this lower clump of cells, we cut additional serial sections from this paraffin block. Only rarely has it been necessary to resort to serial sections. However, originally we generally prepared a short ribbon of sections from each block, cutting, mounting and staining a long enough strip to cover two or three microscopic slides. Thus any suspected group of cells may be followed through for a short distance. In this particular instance, the smaller group, *B*, disappeared in the serial sections, but the larger group *A*, took on a more characteristic appearance of epithelial cells. The next two sections shown in figure 1 (2 and 3) are from this series but are not consecutive. In these sections the polarity of the cells in question is more obvious, and a definite tendency to form an acinus is revealed. There is one definite mitotic figure shown at *C*. Although others have emphasized mitotic figures as of great diagnostic

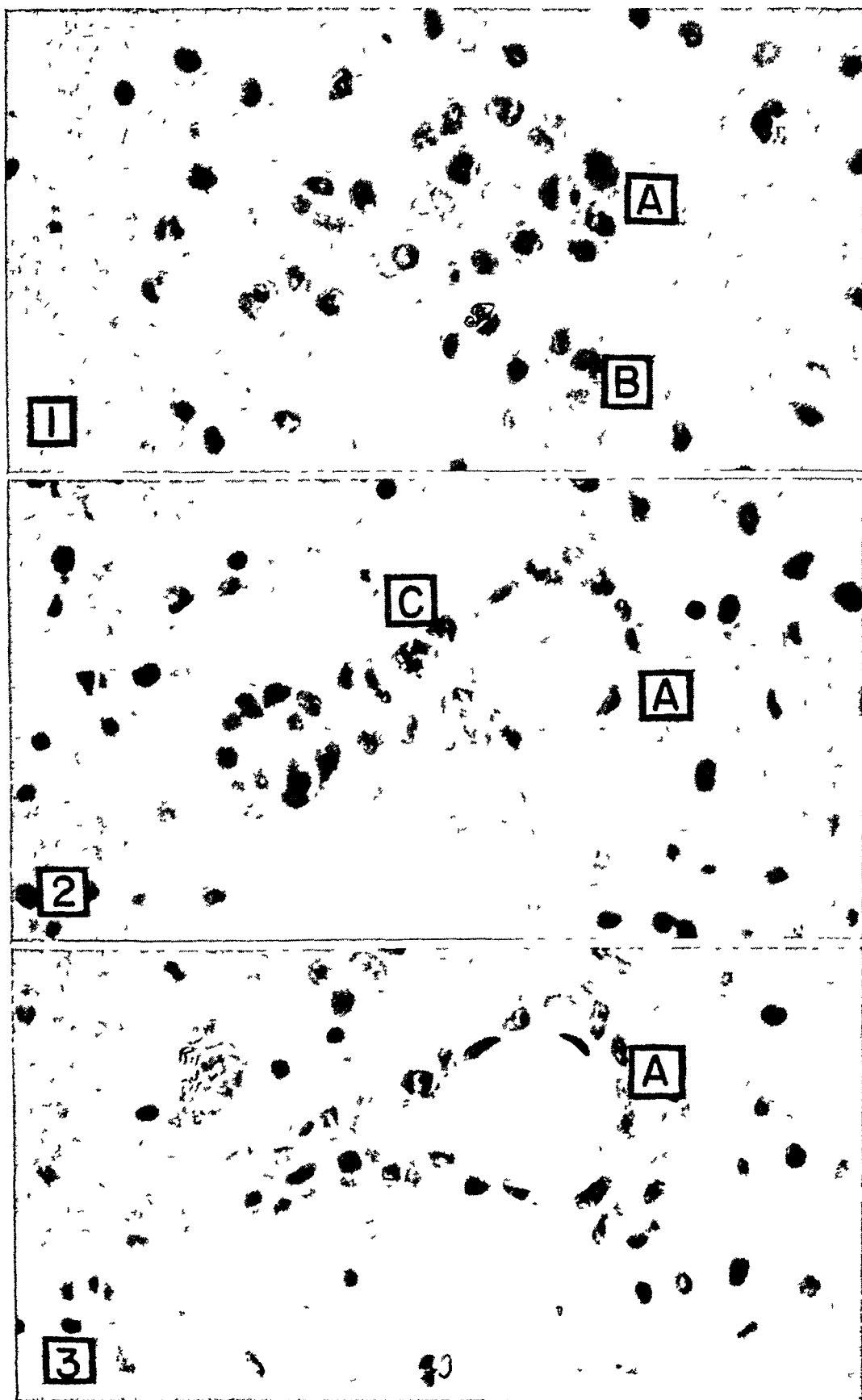


Fig 1 (case 1) —1, 2 and 3, serial sections of cellular sediment from abdominal fluid removed from a patient with carcinoma of the ovary,  $\times 400$



significance in recognizing carcinoma cells, not much reliance can be placed on this finding. In many otherwise most convincing preparations no mitotic figures could be found.

*Comment*—Following the confirmation of the clinical diagnosis of carcinoma by the sections, a laparotomy was performed, and an inoperable carcinoma of the ovary with peritoneal metastases was found.

*CASE 2—Carcinoma of the Ovary* (fig 2 [4])—For two months a 60 year old woman had noticed increase in the size of the abdomen, increase in weight, loss of appetite and development of dyspnea. Examination revealed only ascites. Before tapping the differential diagnosis was cirrhosis of the liver, ovarian cyst or carcinoma. After paracentesis a pelvic mass was palpable. Sections from the sedimented cells of abdominal fluid showed no neoplastic cells, however. A second tap was performed ten days later and the sedimented cells sectioned.

*Sections*—In figure 2 (4), representing this second specimen of fluid, is a definite papillary structure, with the cells arranged about the periphery and demonstrating a special type of polarity. A central stromal core is suggested. In no other fluid have we found anything suggesting a stroma supporting clumps of neoplastic cells, although Graham reported this finding several times.

*Comment*—The patient was discharged with a diagnosis of inoperable papillary carcinoma of the ovary. This was not confirmed by another independent objective method, for no other confirmation of the picture shown was considered necessary. Later a metastasis developed at the site of the paracentesis.

*CASE 3—Carcinoma of the Ovary* (fig 2 [5])—Six months previously the patient, a 49 year old woman, had been operated on for "pus kidney." For two months she had had rectal pain, and for several weeks diarrhea, with bloody stools. For two weeks her abdomen had been swollen. Roentgen examination on admission showed only calcified leiomyoma. The diagnosis on admission was probable carcinoma of the large bowel and splenomegaly. Paracentesis was done.

*Sections*—The sections presented a solid mass of similar appearing cells, the majority of which were probably epithelial cells. From such areas as appear at *A* in figure 2 (5), representing these sections a positive diagnosis of carcinoma could not be made. Similarly at the lower border on the right, at *B*, the cells immediately around what appears to be a large lumen are compressed, have no polarity and are not arranged as a true acinus. At the upper border are three masses of cells more definitely arranged like epithelial cells. The mass at *C* has only a suggestive arrangement. The right hand mass at *D*, however, more definitely resembles an acinus, but most of its cells are indistinct. The left hand mass, at *E*, is undoubtedly half of an acinus. In this fluid the tumor cells were so numerous that a suspicion of carcinoma was aroused from a smear stained with Wright's stain.

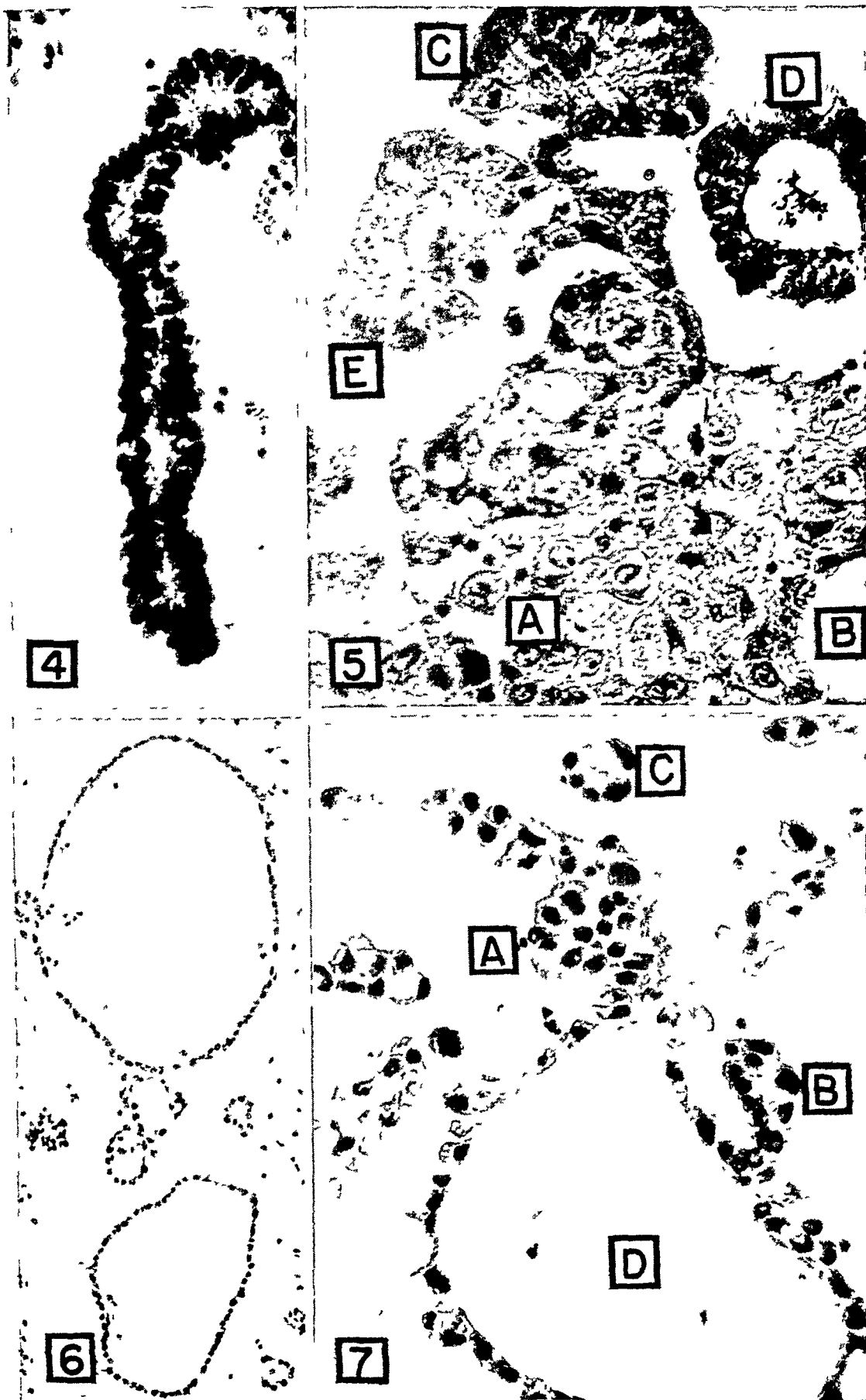


Fig 2—Sections of cellular sediment from abdominal fluid removed from patients with (4) carcinoma of the ovary (case 2),  $\times 200$ , (5) carcinoma of the ovary (case 3),  $\times 200$  (6) carcinoma of the ovary (case 4),  $\times 100$  (7) carcinoma of the uterus (case 5)  $\times 200$

*Comment*—In this patient the sections from the sedimented cells of the fluid established the diagnosis, although the primary site of the carcinoma could not be localized. Usually it is not possible to identify the primary source of the carcinoma cells recognized in these effusions. In this patient an exploratory laparotomy disclosed carcinoma of the ovary with peritoneal metastases.

*CASE 4—Carcinoma of the Ovary* (fig 2 [6])—A 48 year old woman had complained for four months of distention, pain and tenderness of the abdomen and of loss of weight. She was considered to have quiescent pulmonary tuberculosis with probable tuberculous peritonitis. An exploratory operation showed carcinoma of the ovary with multiple peritoneal metastases. Roentgen treatments were instituted. Later there was recurrence of the ascites, and on three occasions fluid was removed and the sedimented cells sectioned.

*Sections*—On all three occasions the sections showed numerous well formed acini composed of cuboid epithelial cells. Several of these acini are shown in figure 2 (6). These present no difficulty in diagnosis.

*Comment*—Probably a preoperative paracentesis with sectioning of the sedimented cells of the fluid would have established the diagnosis and made the laparotomy unnecessary. At all events, the repeated finding of carcinoma cells in the aspirated fluid proved the ineffectiveness of the roentgen treatments.

*CASE 5—Carcinoma of the Uterus* (fig 2 [7])—In a 60 year old woman curettage four months before admission revealed carcinoma of the fundus of the uterus. While she was under treatment for this, her abdomen got larger, and she lost weight. The ascites was relieved by tapping, and the sedimented cells of two specimens of the fluid, removed thirty-five days apart, were sectioned.

*Sections*—Sections representing the first removal of fluid showed many masses of vacuolated epithelial-like cells, most of which were not definite enough to permit an absolute diagnosis of carcinoma. In figure 2 (7), such a mass is seen at *A*, just above the large acinus-like group. However, at *B*, just to the right of the large acinus, is a cluster in which the left hand four cells show the polarity characteristic of epithelial cells. At the extreme top, at *C*, is another group of vacuolated cells arranged as an acinus. The arrangement seen in the large oval acinus, *D*, is never taken except by epithelial cells. The vacuolation of the cells confuses what would otherwise be a picture very easy to interpret. The second examination of fluid showed a few similar groups of cells.

*Comment*—In this case, sections of the sedimented cells of the fluid confirmed the suspicion that the carcinoma of the uterus had already spread to the peritoneum.

*CASE 6—Carcinoma in the Lung* (fig 3 [8])—A 66 year old woman had pain in the lower region of the right side of the chest and loss of weight for one year. Eight days before she was admitted to the hospital, a slight cough and dyspnea developed, with increase in severity of this pain. She was cyanotic and had fluid

in her chest Thoracentesis yielded 1,700 cc of fluid and relieved the pain, dyspnea and cyanosis A roentgenogram made after this tapping revealed definite carcinoma of the lung

*Sections*—The sections of the sedimented cells of the fluid showed innumerable nests of undoubted epithelial cells, everywhere exhibiting polarity and acinar formation, and the diagnosis of carcinoma was made without difficulty At *A* in figure 3 (8) is seen the confusing vacuolation present in some of these cells Some workers have emphasized this vacuolation as a characteristic of epithelial cells, but it is by no means a constant feature

*Comment*—In this instance the sections were merely confirmatory of a finally definite clinical diagnosis, but they served to clinch that diagnosis with a biopsy

CASE 7—*Carcinoma in the Lung* (fig 3 [9])—A 33 year old woman for two months had increasing dyspnea and dry cough Signs of fluid were found over the entire left side of the thorax, and the differential diagnosis was tuberculosis, pleurisy or carcinoma of the lung The chest was tapped, and part of the fluid was injected into a guinea pig, and sections were made from the sedimented cells of the rest Roentgenograms taken after tapping revealed definite carcinoma at the base of the left lung

*Sections*—The sections showed definite carcinoma, but relatively few distinct acini were found In figure 3 (9), in most places the groups of cells only suggest epithelial cell acini Part of one of the two clumps at *A*, however, is definitely one half of an acinus composed of high columnar cells In each of the other clumps, at *B* and *C*, at some place about their circumference a few cells present a definite tendency toward polarity

*Comment*—Here again the fluid made possible a biopsy of an otherwise inaccessible growth and established the diagnosis

CASE 8—*Carcinoma in the Lung* (fig 3 [10])—A 69 year old woman had vague pains in the left side of the chest for two years Shortly before her admission to the hospital flaccid paralysis of the left side developed which had been preceded by pain in the left foot of increasing severity On admission she had signs of a lesion of the spinal cord and of fluid in the thoracic cavity on the right, and the diagnosis of carcinoma of the lung with metastases to the spinal cord was made The chest was tapped, and the sedimented cells of the fluid sectioned, but no carcinoma cells were found Ten days later the cell mass from fluid obtained by another tap was sectioned

*Sections*—Only a few groups of undoubted neoplastic cells were present, but fortunately a group of cells as conclusive as those depicted in figure 3 (10) was found In this group there are (1) acinar formation, (2) polarity, (3) sharply defined cell membranes and (4) a mitotic figure

*Comment*—Repeated roentgenograms, taken both before and after both thoracenteses, were never conclusive, and the diagnosis of a neo-

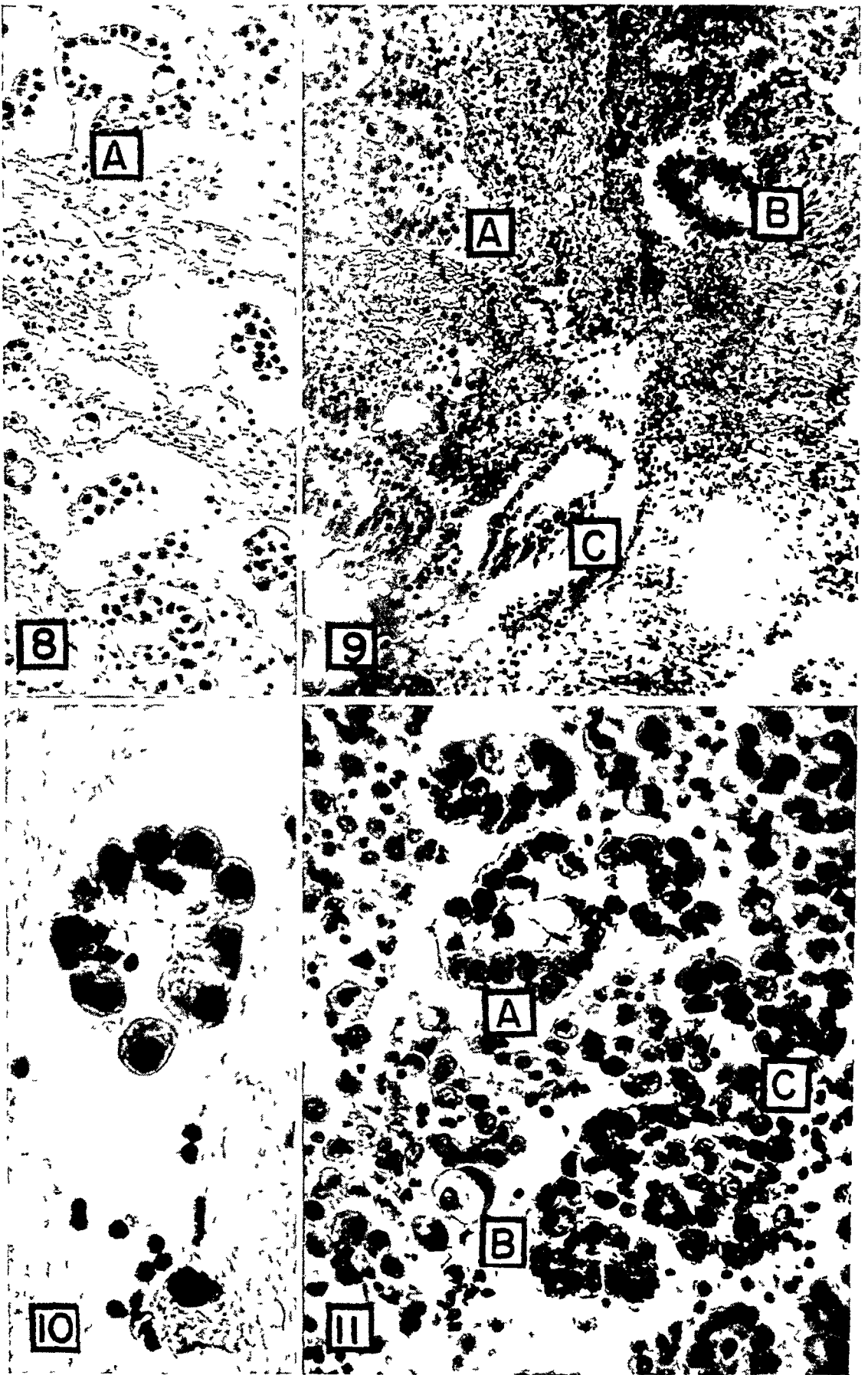


Fig 3—Sections of cellular sediment from thoracic fluid removed from patients with (8) carcinoma in the lung (case 6),  $\times 100$ , (9) carcinoma in the lung (case 7)  $\times 100$  (10) carcinoma in the lung (case 8),  $\times 400$ , (11) carcinoma in the lung (case 9)  $\times 200$

plasm was made entirely on clinical evidence. The sections of the sedimented cells from the fluid were the only objective confirmatory evidence, but they were unequivocal.

*CASE 9—Carcinoma in the Lung* (fig 3 [11]) —For three months a 64 year old man had had pain in the chest, cough, progressive weakness and loss of appetite. He had lost 18 pounds (8 Kg) of weight in three weeks. He showed only clubbed fingers and much fluid in the left side of the chest. The differential diagnosis lay between tuberculosis with effusion and carcinoma. The chest was tapped two days in succession, part of the fluid was cultured, part was injected into guinea pigs, and cellular sediment of the fluid was sectioned on both occasions. The cultures showed no growth of tubercle bacilli and the results of the injections were negative.

*Sections* —On both occasions the sections of the cellular sediment of the fluid showed numerous masses of neoplastic cells. Many acini, more definite than those shown in figure 3 (11), were seen. However, the clump at *A* is clearly enough composed of epithelial cells to establish the diagnosis of carcinoma. The field selected for illustration demonstrates the large number of isolated, less easily recognizable cells between the cell clumps. Many of these separate cells resemble the unquestionable epithelial cells, many are monocytes, many are of doubtful origin. At *B* is a cell phagocytosing a vacuolated cell. Five mitotic figures are shown, four in the lower right hand clump of cells at *C* and one in the clump at *A*.

*Comment* —In this case a roentgenogram made after tapping confirmed the diagnosis of carcinoma of the lung, but the examination of the sections of the cellular sediment from the fluid removed on two occasions served very conveniently as a biopsy to establish the diagnosis.

#### SUMMARY OF RESULTS

Over a period of ten years this method of diagnosis, with use of the technic discussed and of the criteria for recognition of carcinoma cells, has been resorted to in connection with the examination of 145 samples of fluid withdrawn from 100 selected patients. For all these patients the diagnosis of carcinoma was being considered at the time the fluid was withdrawn, and it was hoped that a search for neoplastic cells might be of diagnostic aid. A much larger number of such effusions was withdrawn but not examined by this technic because the presence of carcinoma was either obvious or not suspected. The 145 samples of fluid selected for this type of examination comprised 76 thoracic and 69 abdominal fluids.

As indicated in table 1, some form of carcinoma was the diagnosis at discharge for 60 of these 100 patients and no neoplasm for the other 40. Sections of the sediments from the fluids showed neoplastic cells in 35, or 58.3 per cent, of the 60 cases of carcinoma. The highest incidence of carcinoma cells (100 per cent) was in the 10 cases of carcinoma of the ovary, as would be expected from the well known tendency of this

neoplasm toward peritoneal implantation. However, in 50 per cent or more of the cases of carcinoma in the lung or in the gastrointestinal tract the fluids contained such recognizable neoplastic elements. In only 1 of the 5 cases of metastatic carcinoma of the breast was the condition thus diagnosable, however. A diagnosis was made by this means in a single instance each of carcinoma of the uterus, of carcinoma of the pancreas and of malignant melanoma.

At the time of writing 21 of the 60 patients with carcinoma are alive or cannot be traced. The other 39 died of carcinoma within three years. In only 12 cases was permission for an autopsy obtained, and these autopsies were confirmatory. However, in 19 other cases operation

TABLE 1—Incidence of Carcinoma Cells in Thoracic and Abdominal Fluids

Clinical Diagnosis	Cases	Cases in Which Given Fluid Was Examined		Carcinoma Cells in Fluid			Cases in Which Confirmatory Examinations		Patients Dead at		Patients Alive or Lost
		Thoracic	Abdominal	Cases in Which Cells Were Present	Cases in Which Cells Were Absent	Percent of Cases in Which Cells Were Present	Were Recorded	Were Not Made	1 Yr	3 Yr	
Carcinoma of ovary	10	0	10	10	0	100	9	1	3	4	3
Carcinoma of lung	26	26	0	13	13	50	19	7	13	2	11
Carcinoma of stomach	7	0	7	4	3	57	7	0	4	0	3
Carcinoma of colon	8	0	8	4	4	50	8	0	5	0	3
Carcinoma of breast	5	1	4	1	4	20	4	1	4	0	1
Carcinoma of thyroid	1	1	0	0	1	0	1	0	1	0	0
Malignant melanoma	1	1	0	1	0	100	1	0	1	0	0
Carcinoma of uterus	1	0	1	1	0	100	1	0	0	1	0
Carcinoma of pancreas	1	0	1	1	0	100	0	1	0	1	0
Cases of carcinoma	60	29	31	35	25	58.3	50	10	31	8	21
Cardiac failure	16	13	3	0	16	0	5	11	4	4	8
Cirrhosis of liver	9	1	8	1	8	11.1	5	4	4	4	1
Abscess of lung	7	7	0	0	7	0	4	3	2	1	4
Tuberculosis	4	2	2	0	4	0	2	2	1	0	3
Abscess of liver	1	1	0	0	1	0	1	0	1	0	0
Cyst of liver	1	1	0	0	1	0	1	0	1	0	0
Splenic infarct	1	1	0	0	1	0	1	0	1	0	0
Portal thrombosis	1	1	0	0	1	0	1	0	1	0	0
Cases in which carcinoma was not present	40	26	14	1	39	2.5	20	20	15	9	16

yielded a confirmatory biopsy. In another 19 cases confirmatory objective evidence of carcinoma was obtained by taking an appropriate roentgenogram. Thus, in all but 10 (16.7 per cent) of the 60 cases of carcinoma, the clinical diagnosis was confirmed by another, independent method, such as roentgen study, operation, biopsy or autopsy. In the closely controlled smaller group of 31 cases in which the diagnosis of carcinoma was proved by an independent study of sections of tissue, available because of necropsy or operation or both, there were 20 (64.5 per cent) in which a positive diagnosis was made on the sections of the cellular sediment from the fluid.

In only a single case of a condition not carcinoma, finally shown to be a case of cirrhosis of the liver, were carcinoma cells erroneously

reported as present in the fluid first removed but not in that removed on three subsequent occasions. This erroneous report was made early in the course of this study. In a recent review of the incorrectly interpreted sections it was felt that the same error might be made again by one inexperienced in examining sectioned sediments. Further experience has demonstrated that ascitic fluids from patients with cirrhosis of the liver very often contain groups of large cells which might initially be confused with neoplastic epithelium. More careful study of these cells with use of the rigid criteria discussed in foregoing paragraphs should correct this first impression.

In no other case in which carcinoma was absent was such a diagnosis made on the sectioned sediment of the fluid, although 53 fluids from 40 noncarcinomatous patients were examined. It is important that the criteria and standards finally adopted for any laboratory procedure should be such that the number of erroneous positive reports is reduced to an absolute minimum. There is a definite tendency to place undue emphasis on such presumably objective positive findings. The physician in charge of a patient is less apt to be misled by an occasional erroneous negative laboratory report. If he is still in doubt he may wish to repeat such an examination on a later occasion.

An interesting incidental observation was made that 46 of these 100 patients with ascites or hydrothorax survived less than one year after the fluid had accumulated, and 63 were known to be dead in less than three years. The poor prognosis for patients with fluids in the body cavities, from whatever cause, is thus amply confirmed.

#### REPEATED EXAMINATIONS

From 70 of this series of 100 patients, only fluid removed on a single occasion was available for study, from each of the other 30 patients fluid removed on two or more occasions was centrifuged and the sediment sectioned. Repeated examinations of this type were usually requested by the physician in charge of the patient because the observations on the first examination disagreed with his clinical diagnosis or because the diagnosis was still in doubt. In regard to these 30 patients the final clinical diagnosis was carcinoma for 21 and some other condition for 9.

In table 2 are presented the observations on the fluids from the 21 patients with carcinoma who had such examinations on two or more occasions. In only 4 of the 14 cases of carcinoma in which the result of the first examination of the fluid was negative were carcinoma cells found when the examination of the fluid was repeated. Contrariwise, in 7 cases in which originally the results were positive the results of 4 of the 11 repeat examinations were negative. However, in 2 of the latter cases, in other repeat examinations carcinoma cells were found. Repeat examinations are important and usually helpful when the diagnosis is still in doubt. Sometimes an unequivocal finding will be thus



obtained. At other times the most persistent repetition will not yield a conclusive result. There is a distinct limitation to the method, which must be recognized. As with most properly interpreted laboratory procedures, a negative result may be inconclusive even when repeated, whereas a positive finding should be regarded as of absolute diagnostic significance.

TABLE 2—*Data on Repeated Examinations of Thoracic and Abdominal Fluids from Patients with Carcinoma*

Site of Carcinoma	Fluid Examined	Carcinoma Cells Revealed in Examinations of Fluid							Source of Independent Confirmation
		1st	Interval	2d	Interval	3d	Interval	4th	
Lung	Thoracic	0	6 days	0					Roentgenogram and necropsy
Lung	Thoracic	0	15 days	0					
Lung	Thoracic	0	8 days	0					
Colon	Abdominal	0	4½ mo	0					Operation and biopsy
Colon	Abdominal	0	6 days	0					
Breast	Thoracic	0	5 days	0					Operation and biopsy
Stomach	Abdominal	0	14 days	0	10 days	0			Roentgenogram and necropsy
Lung	Thoracic	0	8 days	0	51 days				Roentgenogram
Lung	Thoracic	0	8 days	0	31 days	0	12 mo	0	
Colon	Abdominal	0	6 days	0	2 yr 60 days	0	8 mo	0	Operation and biopsy
Ovary	Abdominal	0	10 days	+					Operation and biopsy
Lung	Thoracic	0	6 days	+					Operation and biopsy
Lung	Thoracic	0	5 days	+					
Lung	Thoracic	0	10 days	+	28 days	0			
Ovary	Abdominal	+	7 days	0					
Lung	Thoracic	+	1 day	+	34 days	0			
Pancreas	Abdominal	+	9 days	0	10 days	0	7 days	+	Roentgenogram
Uterus	Abdominal	+	35 days	+					Operation and biopsy
Ovary	Abdominal	+	8 days	+					Operation and biopsy
Lung	Thoracic	+	12 days	+					Operation and biopsy
Ovary	Abdominal	+	82 days	+	21 days	+			Operation and biopsy necropsy

#### COMMENT

In making a positive identification of epithelial cells in these sections the most useful characteristic is that the cells in question are oriented as if they had a certain degree of polarity. The arrangement usually is in the form of a whole or a part of an acinus, with or without a lumen. The cells in such a figure generally have their nuclei in the same relative position to the lumen or to the periphery of the acinus, i. e., the cells exhibit polarity. Also, when the cells are so arranged their boundaries are usually quite distinct, and the epithelial cells have a definite polygonal shape, often recognized only after careful focusing under oil immersion.

Occasionally the cells are actually definitely cuboid or columnar. The confusing monocytes and the desquamated serosal cells which usually are also present never have these characteristics. Isolated epithelial cells, however, are often indistinguishable from the latter cells.

The diagnosis of carcinoma by examination of thoracic and abdominal fluids is quite feasible without resorting to any really new principles. The method is not purely objective, however, and it has its limitations. In the final analysis the conclusion hinges on the opinion of the pathologist, on what he sees under the microscope. To be of value, this opinion must be supported by a certain amount of experience, but this experience is easily gained. Other common tests made on exudates, such as those taking account of specific gravity, total cell count, differential cell count, bacteriologic growth and other features, can be and usually are performed by technicians. Frequently the technicians work in a laboratory apart from the laboratories of pathology, where smears of such fluids are readily prepared, but not sections of the sedimented cells. The preparation and examination of sections of exudates for carcinoma cells must be made in the laboratory of pathology. In these sections the pathologist should recognize the microscopic masses of neoplastic tissue as readily as he does groups of tumor cells of similar dimensions in other, more familiar sections. He should not place too much dependence on a study of the minute characteristics of individual cells, no matter how numerous they are. His time will be better spent in a search for and study of groups of cells. He will then study many separate microscopic bits of tissue gathered together into a single section. This is quite analogous to the search in such fluids for pieces of tissue large enough to be seen with the naked eye. Many have commented on the ease of diagnosing a neoplasm by sectioning such relatively large bits of tissue.

#### SUMMARY AND CONCLUSIONS

The diagnosis of carcinoma can be made readily on thoracic and ascitic fluids. Consistent, reliable results can be obtained best by the technic of centrifugation of the fluid and fixation, embedding and sectioning of the sediment. The method of utilizing stained or unstained smears was found to be unreliable.

A definite positive diagnosis of carcinoma should be made on such sections only after finding groups of definite polygonal cells showing polarity, sharply distinct cell walls and acinar or pseudoacinar formation.

Such readily recognizable microscopic bits of tumor tissue are contained in about 60 per cent of the fluids accumulating in the cavities of the body as the result of carcinoma.

The cells of fluids accumulating in the cavities of the body from other causes never assume such a histoid appearance but may otherwise resemble carcinoma cells. If strict adherence to the aforementioned proper, rigid criteria is maintained, a false diagnosis of carcinoma will never be made on such a non-neoplastic fluid.

# CEREBRAL LESIONS IN HYPOGLYCEMIA

## III EXPERIMENTAL INVESTIGATIONS

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MINNEAPOLIS

The continued interest in hypoglycemia as a therapeutic agent has given a definite impetus to investigation of the resulting cerebral changes. The value of a thorough knowledge of the alterations produced in the brain by such treatment is of importance for an understanding not only of the mechanism of the beneficial results of this treatment but also of the dangers that might be involved because of the permanent damage and often continuing destruction of brain

During the past few years there have appeared in the literature sporadic reports describing definite cerebral damage in persons suffering from hypoglycemia (Wolf and others,<sup>1</sup> Morsier and Mozer,<sup>2</sup> Salm,<sup>3</sup> Lindsay and others,<sup>4</sup> Moersch and Kernohan,<sup>5</sup> Kobler<sup>6</sup>) Brain changes of a similar nature have been recorded in the experimental animal Schereschewsky and his co-workers<sup>7</sup> produced acute and chronic insulin poisoning in dogs. The brain tissue of these animals contained numerous old and new hemorrhages as well as many destroyed cortical nerve cells. These changes were scattered throughout the central nervous system but varied in intensity from animal to animal. Stief and Tokay<sup>8</sup> and Grayzel<sup>9</sup> described changes occurring almost exclusively in the nerve cells. Stief and Tokay produced their alterations in 4 rabbits and 4 dogs. The nerve cell changes varied from mild paling of the cell cytoplasm to complete chromatolysis, vacuolation,

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1 Wolf, A, Hare, C C, and Riggs, H. Bull. Neurol. Inst. New York **3** 232, 1933

2 Morsier, G, and Mozer, J J. Ann. de med. **39** 474, 1936

3 Salm, H. Munchen med. Wchnschr. **84** 1046, 1937

4 Lindsay, J W, Rice, E C, Selinger, M A, and Mish, K H. Ann. Int. Med. **10** 1892, 1937

5 Moersch, F P, and Kernohan, J W. Arch. Neurol. & Psychiat. **39** 242, 1938

6 Kobler, F. Arch. f. Psychiat. **107** 688, 1938

7 Schereschewsky, N A, Mogilnitzky, B N, and Gorjaewa, A W. Endokrinologie **5** 204, 1929

8 Stief, A, and Tokay, L. Ztschr. f. d. ges. Neurol. u. Psychiat. **153** 561, 1935

9 Grayzel, D M. Arch. Int. Med. **54** 694, 1934

liquefaction and even pyknosis of the entire cell structure. In the cases of acute poisoning there was no glial reaction, but in the chronic cases there resulted extensive astrocytic proliferation with the formation of glial rosettes. Occasional softened areas were also observed. Grayzel limited his experiments to rabbits, in which he produced repeated hypoglycemic convulsions over a period of three months. The resulting damage to the brain tissue varied from areas of hyperchromatic cells to zones of necrobiosis with mild glial proliferation. Grayzel believed that the severity of the cerebral changes depended on the frequency and intensity of the convulsions regardless of the amount of insulin injected. He concluded that even one convulsion, if severe and prolonged enough, was capable of producing definite lesions within the central nervous system. Dunner and his co-workers<sup>10</sup> and Schmid,<sup>11</sup> experimenting with dogs and rabbits, respectively, also reported cerebral changes after a prolonged series of hypoglycemic reactions. Both investigators emphasized the vascular changes. Dunner was impressed by the extensive endothelial proliferation and new vessel formation. Hemorrhages and foci of encephalomalacia were also observed. Schmid likewise found conspicuous vascular changes in his animals. These were associated with prominent marginal gliosis, diffuse swelling of the astrocytes and definite glial reaction within the basal nuclei. During the past few years further observations on the damage to nerve cells in experimental hypoglycemia have been reported by Tani<sup>12</sup> and Weil and his co-workers<sup>13</sup>. Tani produced, besides these cell changes, increase in glia, sclerosis of the cornu ammonis and softening in the substantia nigra. The severity of the changes did not parallel the number of hypoglycemic attacks, since some animals with very few attacks presented severe damage of the brain. Tani suggested that these differences were influenced by the individual variations in the cerebral vascular supply. Weil and his co-workers were particularly impressed by the alterations in the ganglion cells. They described both acute and chronic changes, the former consisting of liquefaction, vacuolation and homogenization, the latter, of shrinkage and pyknosis. There was definite diminution in the number of neurons throughout the cortex. Proliferation of the macroglia was observed in the more chronic experiments. The vascular changes were mild and were associated with some proliferation of the vascular endothelium. The total dose of insulin used in these animals varied from 28 to 402 units. Weil and his associates concluded that

10 Dunner, L., Ostertag, B., and Thannhauser, S. *Klin Wchnschr* **12** 1054, 1933

11 Schmid, H. *Ann med-psychol* **94** 658, 1936

12 Tani, N. *Zentralbl f d ges Neurol u Psychiat* **80** 30, 1936

13 Weil, A., Liebert, E., and Heilbrunn, G. *Arch Neurol & Psychiat* **39** 467, 1938

the rabbits receiving the larger doses of insulin showed the more extensive histologic changes and that repeated hypoglycemic reactions with small amounts of insulin resulted in little or no damage of the brain, unless the total dose was sufficient to cause such damage

The motivation of the present studies on hypoglycemia was provided by the widespread chronic alterations observed in the cerebral tissues of the human material examined by me<sup>14</sup> It was deemed of distinct value to determine whether these cerebral changes could be consistently reproduced experimentally in animals The chief interest in the present investigations was centered on the chronic and apparently permanent damage of the brain found months after the discontinuation of hypoglycemic shocks The acute changes recorded were only incidental observations in those animals dying unexpectedly during the experiments

#### EXPERIMENTAL INVESTIGATIONS

The 26 rabbits used in these studies were divided into three groups

The 7 rabbits in the first group were subjected to as severe and prolonged a hypoglycemic reaction as they could safely tolerate They were then allowed to live as long as one hundred and thirty days and an attempt was made to determine the influence of a single severe reaction on the brain tissue

The second group contained 13 animals These were given repeated hypoglycemic reactions at weekly intervals over a period of months, the number of total shocks per animal varying from seven to nineteen The rabbits were then allowed to live from one to one hundred days, after which a study was made of the possible damage produced in the brain by these repeated cerebral insults

In the last group were placed 6 rabbits used as controls and kept in the same laboratory as the experimental animals Hypoglycemia was produced by intravenous injection of insulin into rabbits starved for eighteen hours prior to the onset of each experiment The individual dose varied from 10 to 30 units Such a procedure produced very uniform and constant results From one to two hours after the injection the animals began to manifest the first effects of the hyperinsulinism Some would become very quiet and unreactive and shortly afterward would pass into a state of complete flaccidity and coma It was soon learned that the latter condition was very dangerous and required prompt treatment with intravenous injection of dextrose in order to forestall the death of the animal A second type of reaction consisted of the development of moderate or extreme hyperirritability External stimuli would at once produce spasticity of the entire animal After some time these rabbits would begin to manifest convulsive seizures

14 (a) Baker, A. B., and Lufkin, N. H. *Arch Path* **23** 190, 1937 (b) Baker, A. B. *ibid* **26** 765, 1938

lasting about twenty seconds and recurring every ten to twenty minutes. These reactions were not particularly dangerous but were invariably followed by a comatose condition that was very hazardous. The shock was usually discontinued before the onset of this stage.

#### HISTOLOGIC OBSERVATIONS

*Group 1*—Of these 7 animals only a single rabbit failed to recover from the shock therapy. The rest were killed from one to four months later.

**Early Changes** These were limited almost exclusively to the cortical nerve cells, which presented fairly typical acute changes with swelling of the cell body and moderate tigrolysis. Their cytoplasm was usually pale and often vacuolated. The nuclei were, as a rule, intact, although occasionally eccentric in position. More intense damage, when present, resulted in complete fading out of nerve cells to form "ghost cells." When complete destruction occurred, there could be seen large clear spaces containing an occasional cytoplasmic fragment or an isolated nucleus but no demonstrable cell body. These acute and subacute changes were generalized, being at no time limited to any single region. The injured cells were usually interspersed among the unharmed and structurally normal elements. Glial changes were not observed.

**Chronic Changes** The damage observed in the brains of those animals which lived for long periods after the single cerebral insult was much less striking than the acute changes. Most of the nerve cells appeared intact. There were a few shrunken, irregular cells within the cerebral cortex. These were markedly pyknotic and contained irregular clumped cytoplasmic granules, tortuous and often fragmented processes and eccentrically placed irregular nuclei.

The macroglia presented mild proliferation. They were moderately increased in number, but their nuclei remained fairly uniform in appearance. This glial proliferation was scattered throughout the brain tissue and often occurred in small localized areas to form tiny glial nodules.

Petechial hemorrhages were not uncommon. These bleedings were discrete, of a ball type and limited primarily to the white substance in the vicinity of the blood vessels. None of these hemorrhages appeared to have caused destruction of the adjacent brain tissue.

#### COMMENT

The cerebral changes resulting from a single severe prolonged hypoglycemic reaction varied, depending on the stage at which the tissue was studied. Shortly after recovery from the shock there occurred acute swelling of the nerve cells. This early cell reaction appeared to be reversible and tended to disappear in a short time. A few cells, however, were more severely injured and degenerated into a stage of chronic alteration and nonfunction. A reaction on the part of the glial elements was found only as a chronic process but was never conspicuous in these cases of single cerebral insults.

*Group 2*—As stated previously, the number of hypoglycemic shocks administered to these animals ranged from seven to nineteen, while the total time that elapsed from the onset of treatment until the brain was removed for study varied from forty-nine to one hundred and ninety days. One hundred and ninety

days seemed an adequate period for the appearance of all possible types of permanent cerebral damage

The alterations in the nerve cells were not conspicuous. As a rule some cells could be found that were shrunken and free from Nissl substance. Their processes were narrowed and irregular, and the entire cell seemed to be collapsed around an irregular, pyknotic nucleus. These changes were scattered with no tendency to localize in any special region of the brain. In a rabbit that had obtained nineteen hypoglycemic reactions the cells in many cortical areas had undergone extensive fragmentation, liquefaction and even actual disappearance. Peculiarly enough, the adjacent cortical cells were intact. The damage of the nerve cells seemed to parallel the number of cerebral insults suffered by the animals, those with the greatest number of attacks of hypoglycemia presenting the most severe chronic alterations of the nerve cells.

The macroglia presented some of the most striking changes. These varied from mild diffuse increase to extensive proliferation, with extreme variations in the size and shape of the young astrocytes (see *A* in figure). Frequently this glial increase remained well localized, producing subpial or perivascular gliosis. The latter was the more common and in some regions obliterated the vessel wall and extended into the adjacent brain tissue. Focal increase in the macroglia unassociated with vessels was also very common. These glial nodules varied in size from some composed of only a few proliferating astrocytes to others containing an extensive cellular reaction covering large areas of tissue. The macroglial reactions appeared to have very little relationship to either the number of hypoglycemic reactions or to the quantity of insulin injected into the animals. It was quite apparent that the macroglial changes were much slower in onset than those of the nerve cells and that sufficient time had to be allowed for them to become apparent.

Injury to the myelin sheaths was also prominent. The milder lesions consisted of focal or diffuse demyelination with accumulation of microglia around and within these softened areas. Many regions had completely lost their tinctorial properties, although actual demyelination was not yet apparent. In the more advanced lesions complete destruction of the tissue had occurred, producing many small irregular cavitations (*B* in figure). These were occasionally lined by glia and often contained red cells that had extravasated from adjacent capillaries. In some areas there occurred very irregular myelin degeneration, giving to the brain tissue a peculiar vacuolated appearance (*C* in figure). In these regions the astrocytes occasionally proliferated to replace the destroyed areas. As in the previous brain changes, the severity and extensiveness of the myelin injury did not seem to be related either to the number of hypoglycemic reactions or to the amount of insulin given the animals.

Vascular changes of some type were observed in almost every case. Vascular congestion was conspicuous. Hemorrhages were an almost constant finding and varied from a few perivascular erythrocytes to extensive bleedings that destroyed brain tissue and occasionally tore through the cortex into the subarachnoid space. The petechiae were almost exclusively of a ball type and usually remained discrete, with little or no injury to the surrounding substance (*D* in figure). Larger hemorrhages were unusual but when present invariably destroyed some of the adjacent brain tissue and produced fairly large areas of surrounding demyelination (*D* in figure). Hemorrhages also occurred within the cortex, where the larger ones often extended through into the subarachnoid space. In some of the bleedings the red cells had already undergone complete hemolysis with the formation of a homogeneous mass. Hemosiderin granules could also be found scattered in some



EXPLANATION OF FIGURE

*A*, mild diffuse increase of the macroglia, hematoxylin and eosin stain,  $\times 200$ . The cell nuclei are fairly uniform in size and shape. There is beginning destruction of the myelin tissue.

*B*, portion of an irregular cavitation observed within the brain of a rabbit subjected to repeated hypoglycemic shock, hematoxylin and eosin stain,  $\times 200$ . A few erythrocytes are seen in the wall of the cavity.

*C*, an area of brain tissue demonstrating irregular degeneration of myelin, hematoxylin and eosin stain,  $\times 300$ . The entire tissue is undergoing degeneration, producing a peculiar vacuolated appearance. Even the cell bodies are involved and cannot be seen clearly.

*D*, petechiae in the brain of a rabbit subjected to repeated hypoglycemic attacks, hematoxylin and eosin stain,  $\times 300$ . The hemorrhages are mostly of a ball type and are frequently composed of very dense masses of erythrocytes. Note the beginning demyelination of some of the adjacent brain tissue.



of the injured brain tissue. The quantity of hemorrhages varied from animal to animal. They tended to be most common in those rabbits that had had the greatest number of hypoglycemic shocks.

#### COMMENT

These histologic studies of the nervous system of rabbits exposed to varying degrees of hypoglycemic shock indicate that such treatment produces widespread severe damage of the brain, which is even more marked in the chronic phases of its development than in the acute stages. In the chronic stages of such injury the nerve cell changes are not conspicuous but are surpassed in intensity by the cerebral petechiae, the areas of demyelination and the diffuse and focal glial proliferation. Chronic cerebral tissue alteration of a similar nature has already been mentioned in sporadic cases in man (Lindsay and others,<sup>4</sup> Hartman,<sup>15</sup> Salm,<sup>3</sup> Morsier and Mozer,<sup>2</sup> Baker<sup>14b</sup>). Any single one or any combination of these alterations is quite capable of producing severe lasting injury of the brain.

The question arises as to the explanation of such extensive damage of the brain. Weil and his co-workers were of the opinion that the total dose of insulin and the severity of the cerebral injury were in some way closely related. Such a correlation was not observed in the present studies. A more likely explanation might be found in the frequency and severity of the possible cerebral anoxemia produced by the hypoglycemic reactions. Impaired nutrition of the brain must accompany hypoglycemia. It is well known that cerebral changes follow circulatory disturbances due to impairment of cerebral circulation. Such alterations have been described in man by Doring<sup>16</sup> and Kammy<sup>17</sup>. In persons dying from obstruction of the cerebral circulation they observed injury of nerve cells, many small diffuse areas of demyelination and focal areas of glial proliferation. Similar changes were produced experimentally in cats by Gildea and Cobb<sup>18</sup> by ligation of the carotid arteries. In hypoglycemia it is probable that a similar circulatory disturbance results, only here it is of a qualitative nature inasmuch as the blood reaching the cerebral tissues is deficient in the proper nutritive materials.

#### SUMMARY

Rabbits were subjected to repeated hypoglycemic reactions and their nerve tissues studied months after the last reaction in order to evaluate the resulting chronic and permanent damage of the brain.

15 Hartman, F. W. *J. A. M. A.* **109** 2116, 1937.

16 Doring, G. *Virchows Arch. f. path. Anat.* **296** 666, 1936.

17 Kammy, E. *Beitr. z. path. Anat. u. z. allg. Path.* **100** 248, 1938.

18 Gildea, E. F., and Cobb, S. *Arch. Neurol. & Psychiat.* **23** 876, 1930.

Although nerve cell changes did occur, these were by no means as striking as the cerebral hemorrhages, the areas of demyelination and encephalomalacia and the glial reactions

No correlation could be found between the total dose of insulin administered and the severity of the damage of the brain

It is suggested that the cerebral damage in hypoglycemia might be due to a qualitative circulatory disturbance inasmuch as the blood reaching the brain is deficient in the proper nutritive materials

Federal Aid students assisted in the care of the animals during these experiments

# NEW FORMATION OF ELASTIC TISSUE IN ADHESIONS BETWEEN SEROUS MEMBRANES AND IN MYOCARDIAL SCARS

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Some years ago, in collaboration with Dr Gaylord Coon, I made some preliminary experiments in an attempt to find under what conditions elastic tissue might be laid down in repair tissue. These experiments went only so far as the demonstration that in the scar tissue that resulted from the healing of ulcers of the skin of rabbits there was no new formation of elastic tissue. There was, however, slight evidence of growth of the cut fibers at the edges of the wounds in the form of a brushlike extension of short delicate fibrils from these fibers. The only other positive observation in the series of sections was that the youngest connective tissue fibers, i e, the ones immediately around the fibroblasts in the developing repair tissue, were argentaffin in specimens stained with the Bielschowsky silver stain, while the more distant, older fibers took the orange-yellow stain of collagenous tissue.

At that time the idea was present in our minds, from the distribution of elastic tissue in the body and from its new formation in such locations as the intima of an artery, the seat of endarteritis, that if one could subject developing connective tissue to protracted alternations of tension and relaxation, the development of elastic fibers might be obtained. However, an appropriate experimental setup did not present itself at that time, and the study was dropped.

Recently it occurred to me that in a variety of pathologic processes in man nature has performed the desired experiment, and this has led to the present study. The experiment par excellence is that in which vascularized fibrous adhesions have resulted from the organization of an adhesive fibrinous exudate in a serous cavity. Such connective tissue strands, joining the two layers of the pericardium, are subject to variation in tension with every heart beat, and those of the pleura, with every respiration. Myocardial scar tissue, likewise, is subjected to variations in tension during the cardiac cycle.

The first case studied was that of a woman of 46 who gave a history of rheumatic fever at the age of 10 years and "heart trouble" from

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that time on. At postmortem examination there were found, in addition to valvular lesions and a hypertrophied heart (480 Gm), a scarred myocardium and chronic fibrous adhesions between the parietal and the visceral layer of the pericardium over the left ventricle and left auricle. Histologic sections of these adhesions stained by the Weigert elastic tissue stain show a well marked development of elastic tissue (fig 1). There is a considerable network about the new blood vessels and adjacent to them, but also heavy strands, independent of the blood vessels, are found extending in a line parallel to the line of tension in the adhesion. While much of this has the sharp double contour of active, healthy elastic fibers, in many places there is evidence of degeneration. The fibers are broken and granular and present a fuzzy appearance. The visceral pericardium itself shows a marked increase in elastic tissue beneath the adhesions. There are heavy fibers not only in the submesothelial fibrillar tissue but also in the deeper layers of the membrane and even among the adipose tissue cells.

Sections of a myocardial scar in the same heart showed, with the Weigert elastic tissue stain, an extremely dense development of heavy elastic fibers running parallel to one another and to the intervening collagenous fibers and adjacent muscle fibers. The fibers were so numerous and so closely packed that the specificity of the stain was under suspicion until close observation showed at the edges the characteristic doubly contoured branching fibers (fig 2B).

These specimens are not unique. Figure 2A is from an elastic tissue stain of the scar of an old infarct in the 1,060 Gm heart of a 54 year old man with generalized arteriosclerosis, including marked involvement of the coronary arteries. The section is cut somewhat obliquely to the direction of muscle and scar tissue but indicates perhaps better the typical morphologic picture of the cut heavy elastic fibers than a directly parallel section.

In all other cases of adherent pericardium and of myocardial scars examined, new formation of elastic fibers has been found. Such new growth is present not only in fibrillar adhesions between the serous surfaces but also in diffuse or universal adhesions. In all cases the direction of the main elastic fibers has been parallel to the apparent line of tension, i. e., parallel to the connective tissue bundles or to the heart muscle fibers.

In the fibrous thickening of the endocardium of the walls of dilated chambers of the heart there is also an increased elastic fiber content, but in this location it is more difficult to determine that it is not the result of a development from the preexisting normal elastica of the endocardium.

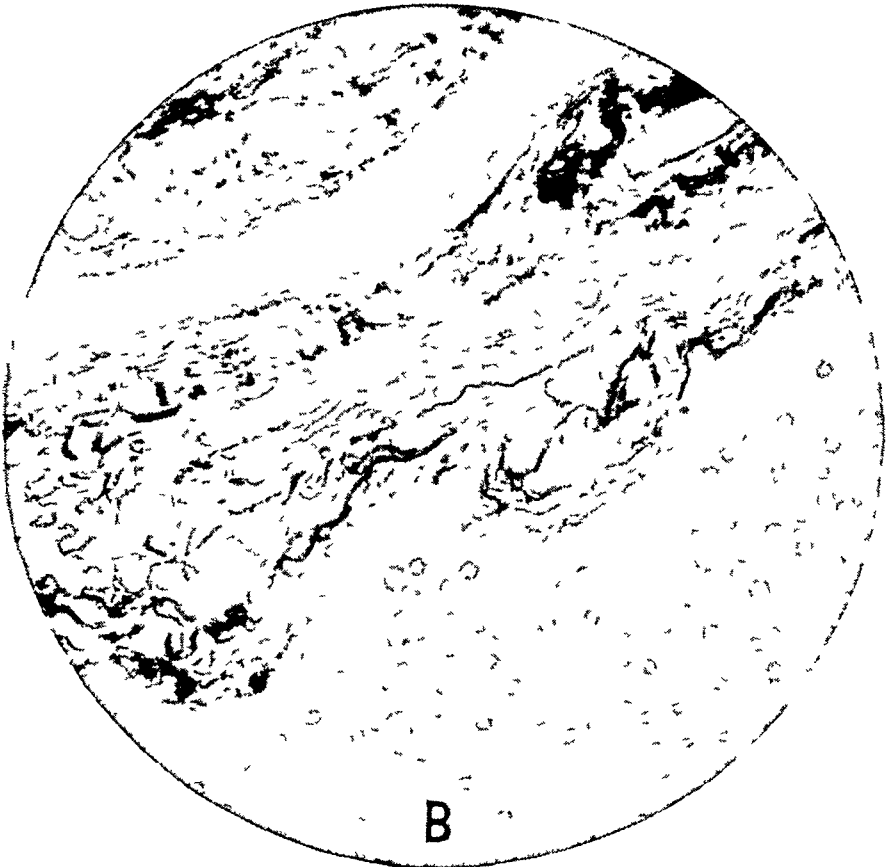
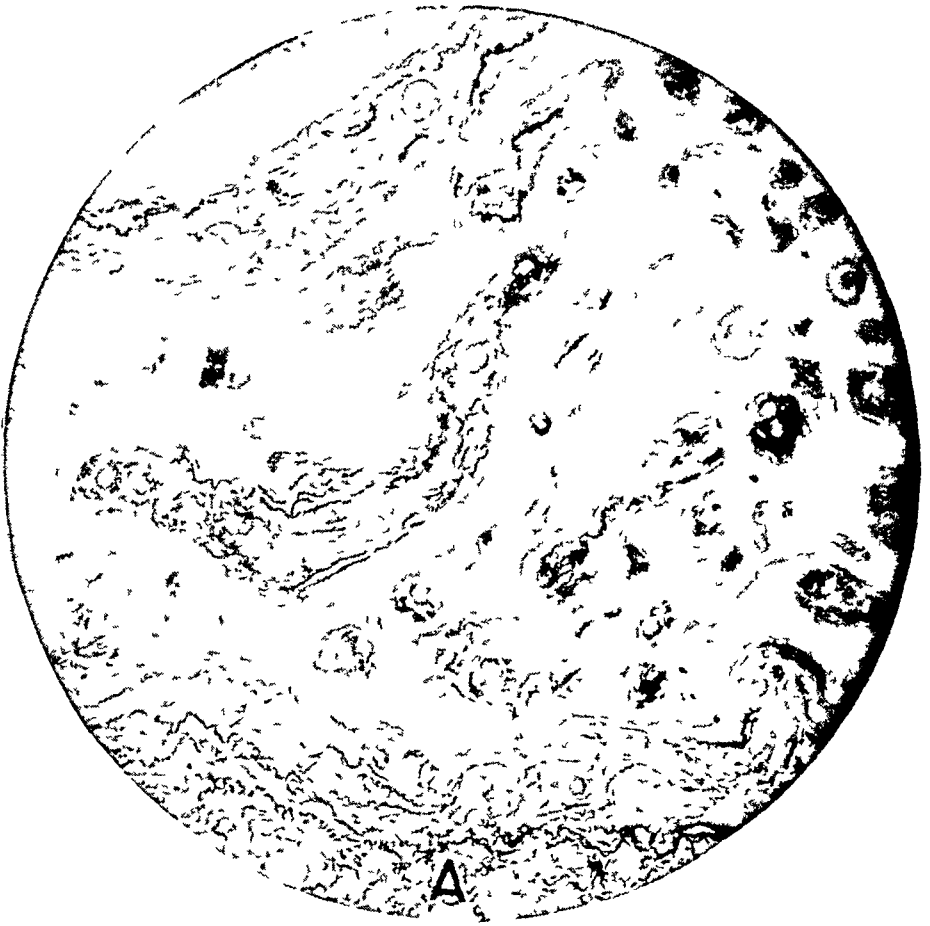


Fig 1—*A*, low power photomicrograph of visceral pericardium and fibrous adhesions, *B*, high power photomicrograph of a single adhesion in the same case, Weigert elastic tissue stain

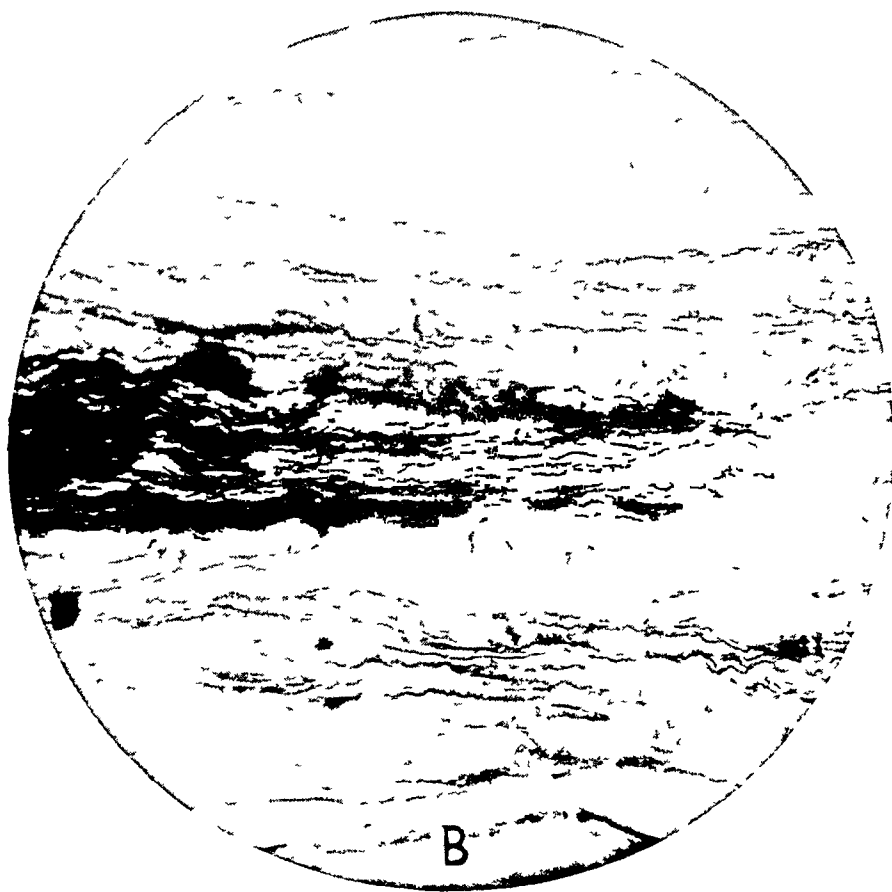
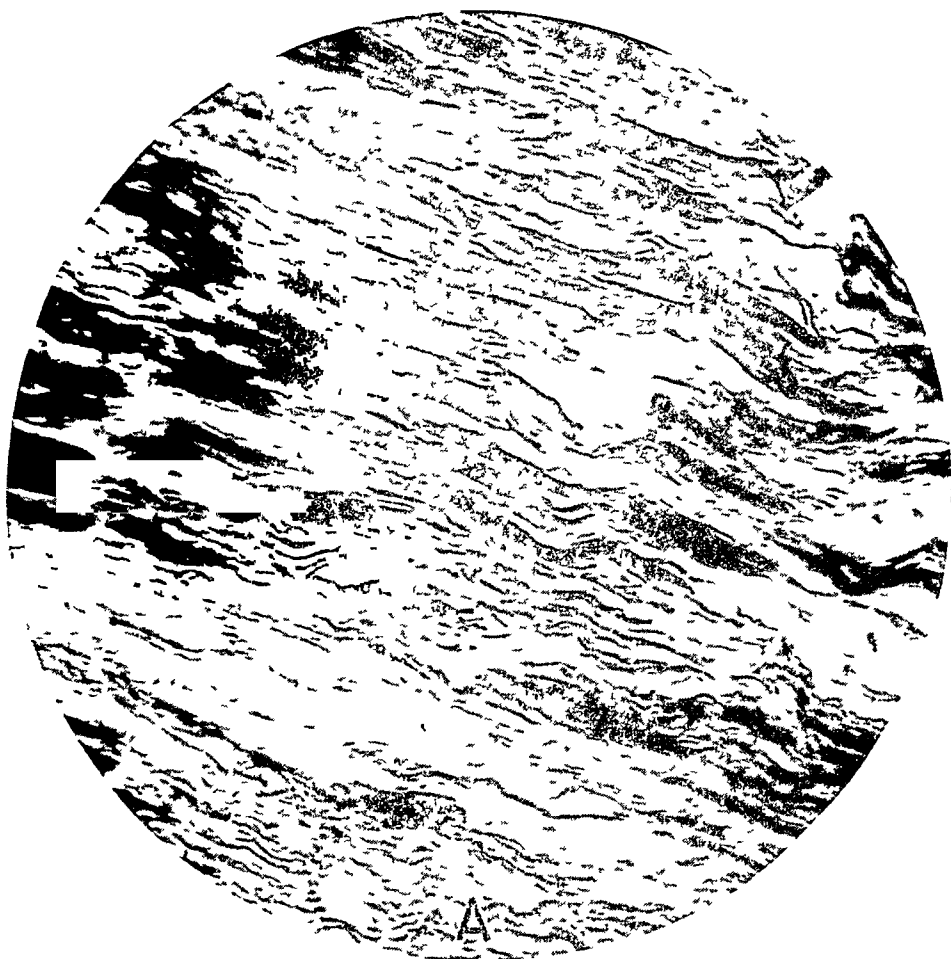


Fig 2—*A*, photomicrograph of old scar of a cardiac infarct, Weigert elastic tissue stain *B*, edge of myocardial scar in the same case as tissue shown in figure 1

A similar development of elastic tissue, although somewhat less extensive, has been found in all pleural adhesions which have been examined, both the diffuse and the fibrillar type. The new fibers are found not only in connection with the new capillaries, among the older of which they form a definite layer of the vessel wall, but among the collagenous fibers and to a large extent definitely parallel to them. From previous



Fig. 3—High power photomicrograph of chronic fibrous pleural adhesions, Weigert elastic tissue stain

experimental work this appears to indicate that they are laid down parallel to the line of tension<sup>1</sup>. In figure 3 is reproduced a typical picture of pleural fibrillar adhesions stained by the elastic tissue stain. At the left of the photograph it may be seen that the elastic fiber of the adhesion is continuous with a newly developed elastic fiber which lies outside of the elastica propria of the visceral pleura and is unconnected with it.

1 Bunting, C. H., and Eades, C. C. *J. Exper. Med.* **44**, 147, 1926

A feature of certain pleural adhesions which deserves further study is a new growth of smooth muscle both in the visceral pleura and in the adhesion itself. In the pleura the muscle cells appear to be associated chiefly with the lymphatics. In the adhesions some bands of muscle are in association with newly formed vessels and others are quite independent of them.

In a fibrillar adhesion between the spleen and diaphragm a definite but not extensive new formation of elastic tissue was found.

Checking these findings with old repair tissue in man, one finds that where the latter is not subject to any marked intermittent strain the findings agree with those in the rabbit. Elastic tissue is not laid down among the collagenous bundles. There may be some new tissue of elastic type laid down in association with the new vessels, parallel to them, and usually perpendicular to the new connective tissue.

Whether or not these findings are sufficient to establish the thesis that tension, and probably intermittent tension of the tissue, is the factor that determines the formation of elastic tissue in repair may be questioned. The development of elastic tissue in the lung, however, seems to follow the establishment of respiratory activity if one may accept as fact that there are intrauterine respiratory movements. In the lungs of a 6.5 cm fetus there was well developed elastic tissue only in the vessels with a few delicate fibrils beginning to show in the walls of the trachea and larger bronchi. In 2 fetuses 30 cm in length there were a delicate submesothelial line of elastic fiber in the pleura, a subepithelial layer in the larger and medium-sized bronchi and few dots and short delicate fibrils (best brought out by an orcein stain) in the respiratory bronchioles and ductuli alveolares. In 2 fetuses of 41 and 42 cm length, the pleural elastic layer consisted in the main of a single layer, but in some places it was doubled. Within the lung the musculoelastic layer of the respiratory bronchioles was sharply developed, their alveoli, however, showed no elastic tissue in the walls.

This condition persists until the time of birth, and, in fact, until some months after birth—for it is only in children 7 months old that I have found the beginning of elastic tissue in the alveolar walls, and only in children 10 months old that it is well developed. In this same interval the elastica propria of the pleura has become a double or even triple layer structure with interlacing fibrils. It seems, then, that elastic tissue in the lung is developed only with increasing activity of that organ.

It was not the purpose of this study to try to answer the old question as to whether elastic tissue is laid down as such by connective tissue cells or whether preexisting collagenous fibers are converted into elastic tissue. I doubt that the specimens studied can give a satisfactory answer. One may convince oneself from the relation of the elastic fibers to



collagenous bundles in an old myocardial scar that the elastic fibers must be due to a conversion of the collagenous tissue and then be confronted with a more recent pleural adhesion in which terminal edema had widely separated the elements and in which the new elastic fibers are so closely applied to the young fibroblasts that it seemed that they must be a direct result of cellular activity. It is, of course, possible that elastic fiber is formed by both methods.

In conclusion I would note that in adhesions between serous membranes and in scars, where both are subject to alternations of tension and relaxation abundant elastic tissue is developed with its main direction parallel to the line of tension. The excess development in such areas in contrast to that in scar tissue in other locations seems to indicate the importance of this mechanical stimulus to its development. Other factors are of course not excluded.

# HISTOLOGIC STUDY OF REPARATION OF EXPERIMENTALLY PRODUCED DEFECTS IN CALVARIUMS OF RATS

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That fractures and defects of the calvarium heal slowly or not at all has been a frequent observation. Most of the reports in the literature are based on clinical investigations in man<sup>1</sup>. The lack of exact knowledge concerning the processes involved seemed to warrant an experimental investigation into the matter.

The study was conducted in the following fashion:

Two groups of albino rats, unselected for sex and averaging, respectively, around 80 and 150 Gm in weight, were subjected to operation. With the rat under amytal anesthesia, a midline sagittal incision was made in the skin over the calvarium. The point of the scissors was pressed through the parietal bone on one side, with as little damage to the brain as might be, and a triangular piece of bone measuring approximately 3 mm on each side was removed. The skin was then sutured. The animals were kept on stock diets and killed at the periods of time after operation shown in the table.

For the histologic examination, the head was removed, sawed partly in half along the midline and fixed in Zenker's solution, and then both sides were sectioned and the sections stained with hematoxylin and eosin.

## OBSERVATIONS

*Young Rats (80 Gm)* —It was found that a few fragments of bone were left as a result of the operative procedure. There was a considerable amount of hemorrhage but only superficial destruction of the

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\*Frauenthal Orthopedic Travel Fellow (1937)

From the Laboratory Division, Hospital for Joint Diseases

1 Meade, R H. *Med-Chir Tr* **23** 390, 1840. Watson, A. *Edinburgh M J* **63** 302, 1845. Weist, J K. *Cincinnati Lancet & Obs* **9** 155, 1866. Shoemaker, G E. *Philadelphia M Times* **13** 745, 1883. Billroth, T. *General Surgical Pathology and Therapeutics*, translated and revised by C E Hackley, New York, D Appleton and Company, 1886. Helferich, H. *On Fractures and Dislocations*, New York, William Wood & Company, 1899. Koenig, F. *Lehrbuch der speciellen Chirurgie*, Berlin, A Hirschwald, 1875, vol 1, p 38. Macewen, W. *The Growth of Bone*, Glasgow, J Maclehose & Sons, 1912. Naffziger, H C, and Glaser, M A. *Surg, Gynec & Obst* **51** 17, 1930. Wakeley, C P G. *Practitioner* **127** 75, 1931. Ireland, J. *Arch Surg* **24** 23, 1932. Troitzky, W. *Ztschr f Morphol u Anthropol* **30** 504, 1932. Wright, L T, Greene, J J, and Smith, D H. *Arch. Surg* **27** 878, 1933. Echlin, F. *ibid* **28** 357, 1934. Earley, D E. *J A M A.* **104** 2332, 1935.

bium. None of the animals exhibited obvious neurologic defects. The hemorrhage was quickly resorbed. After a lapse of eight days the gap was closed by a membrane of usually not very cellular fibrous connective tissue. There was localized necrosis of the fractured ends. The pericranial membrane had undergone proliferation to a moderate degree, as had the dura. Both lateral sides of the skull showed this change. At this early period ossification had already commenced in the periosteum near the fractured edge but was exceedingly limited in extent. The pericranium was the more active in this phenomenon. By the thirteenth day the corners of the defect had been completely bridged by new bone, while the wider part had granulation tissue only (fig 1 A).

In the eighth week newly formed fiber bone had reduced the cavity by half. Near the defect there was little periosteal activity on either the

*Interval Between Experimental Production of Defect in Rat's Skull and  
Histologic Examination of Repair of Defect*

Young Rats (80 Gm )		Adult Rats (150 Gm )	
Rats	Interval	Rats	Interval
1	4 days	1	4 hours
1	8 days	1	2 days
1	13 days	1	4 days
1	53 days	3	8 days
1	80 days	1	11 days
1	90 days	2	14 days
1	140 days	1	17 days
1	150 days	1	21 days
1	180 days	1	30 days
2	300 days	1	150 days
		1	175 days
		1	210 days
		1	300 days

outside or the inside of the calvarium. After five months the site of operation was no longer identifiable.

*Adult Rats (150 Gm )*—The initial appearance of the lesion was similar to that already described. By the fourth day there had been some pericranial proliferation on both lateral sides of the skull. On the eighth day also the two pictures were very similar (fig 1 B). In different rats the dura or the pericranium might assume the main burden of bony replacement. One case, indeed, was noted in which there was already considerable new bone in the defect.

In the ensuing weeks the picture changed slowly (fig 2 A). Shelves of newly formed bone appeared on the fracture edges and began to bridge the gap. This was usually a markedly asymmetric process, one side participating hardly at all (fig 2 B). Canalization of the new bone had begun.

In 2 rats examined fourteen and seventeen days after operation, respectively, buttons of excised bone had inadvertently been left in place. These were serving as scaffolds for osteogenesis—in one rat, by the periosteum of the fragment, and in the other, by invasion of the diploe from the broken ends (fig 3). A thirty day period brought about no advance of the process.

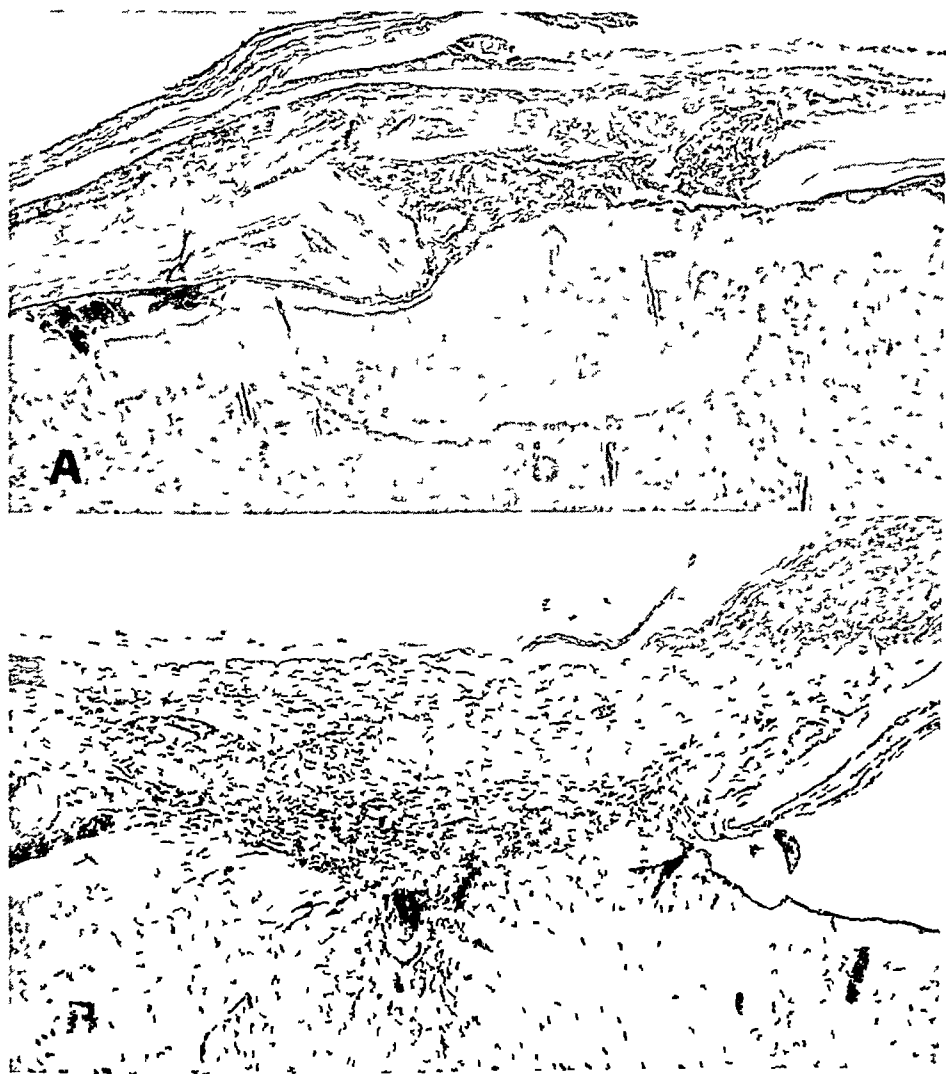


Fig 1—*A*, photomicrograph showing the narrower part of the defect bridged by new bone,  $\times 30$ . Note (arrow) the newly formed dural membrane. Brain is seen in the lower half of the picture (*b*). *B*, photomicrograph showing a densely cellular mass of fibrous connective tissue filling in the gap after an interval of eight days,  $\times 20$ .

Some of the histologic preparations of animals killed at one hundred and seventy-five days showed the defect still present and bridged only by a rather poorly cellular connective tissue membrane. The pericranium

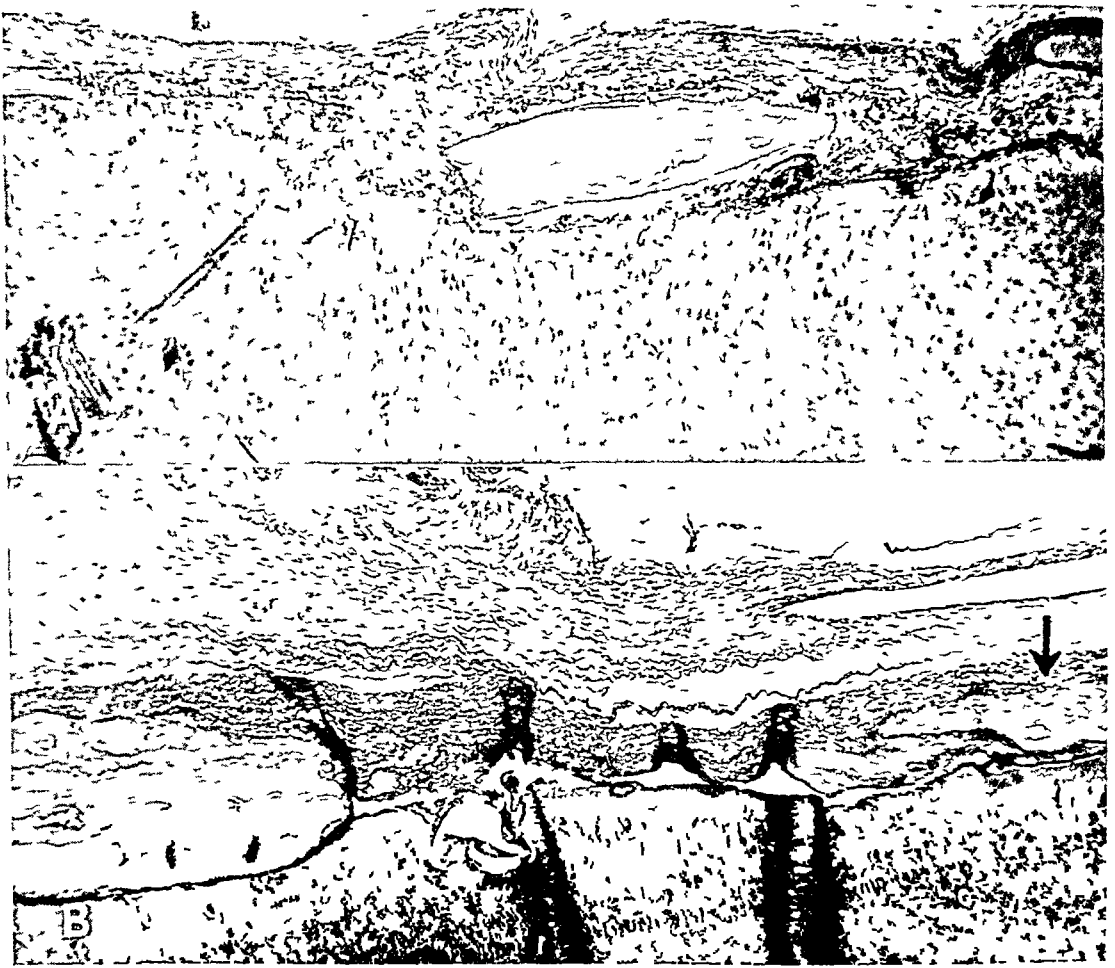


Fig 2—*A*, photomicrograph showing absence of osteogenesis in the calvarial gap after fourteen days,  $\times 30$  Note the free piece of necrotic original calvarial bone *B*, photomicrograph revealing a tongue of fiber bone spreading across the defect (arrow) after an interval of eleven days,  $\times 20$

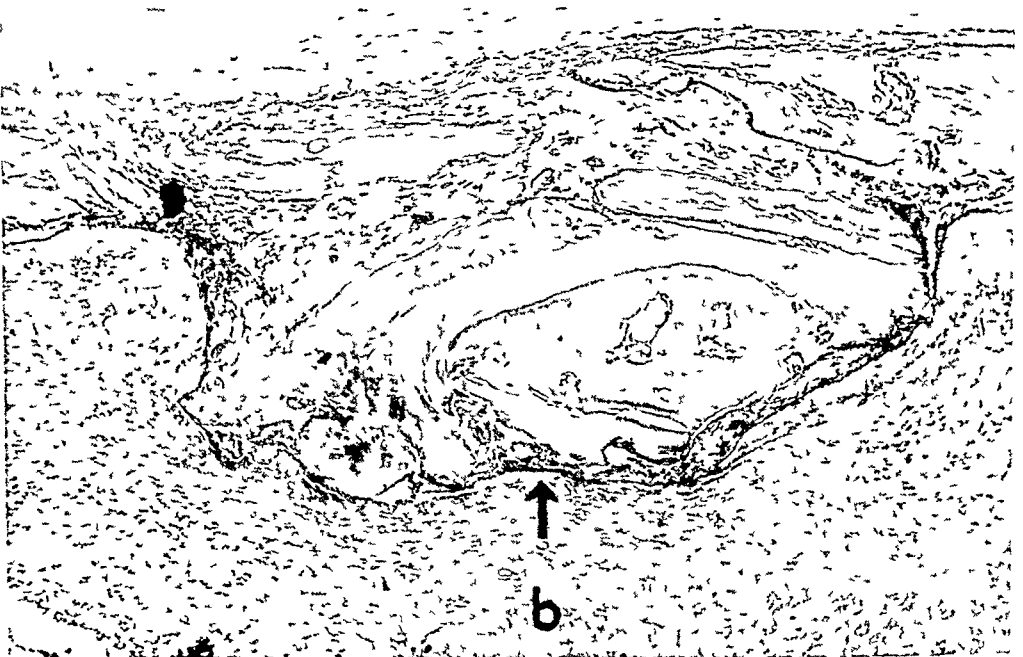


Fig 3—This photomicrograph reveals new bone formation about the depressive fragments of calvarium,  $\times 30$  Note again the realignment of the newly formed dural membrane (arrow)

and dura were quiescent and were being restored in the defect. There had been thickening of the fractured edges with healing in some necrotic fragments. Other slides, probably from narrower portions of the incisions, showed complete bony continuity, with excessive bone on the inner side.

It is noteworthy that nowhere was true callus encountered. There were indeed a very few feeble attempts at metaplastic ossification on the part of the connective tissue which closed the wound in the calvarium. Nowhere, however, did this reach an extent or vitality comparable to that commonly seen after fracture of a long bone. Indeed, it was for the most part so sluggish in appearance, so poorly cellular and ill vascularized that it hardly merits the appellation of granulation tissue. It is perhaps worth mentioning that cartilage did not develop. On the contrary, where bony bridges appeared, they seemed to be the result of a steady progression, probably through the intermediation of osteoblasts from the broken edges, outward across the opening. Infection occurred in a few instances and seemed to exercise a depressing influence on the healing process. Only once was it severe. The finding of bony thickening of the fracture edges may be correlated with the occasional roentgenographic observation of increased density along the old fracture lines in man.<sup>2</sup>

#### SUMMARY

The reparation of experimentally produced defects in the calvariums of rats was slow. In the young rats the defects were reduced by newly formed fiber bone to only one half of the original area after a period of eight weeks. In the older rats the defects were noted even after one hundred and seventy-five days.

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<sup>2</sup> Stewart, W. H. *Brit J Radiol* **30** 399, 1925. Illeg, W. *Fortschr a d Geb d Rontgenstrahlen* **43** 76, 1931. Lindemann, E. *Arch f Ohren-, Nasen- u Kehlkopfh* **135** 25, 1933. Glaser, M. A., and Blaine, E. S. *J A M A* **107** 21, 1936. Vance, R. G. *Am J Roentgenol* **36** 744, 1936.

# CHEMOTROPISM OF HUMAN EOSINOPHILIC POLY-MORPHONUCLEAR LEUKOCYTES

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AND

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Although a great deal is known about the function of neutrophilic leukocytes in inflammation, this is far from being true in regard to polymorphonuclear eosinophils. As far as can be determined by search of the literature it is not known whether these cells in man show chemotaxis either to bacteria or to those animal parasites which cause eosinophilia. Rosegger<sup>1</sup> reported that *Ascaris* attracts eosinophils obtained from horse blood. His report is based on a cinematographic study of the behavior of a very few cells, and the results cannot be analyzed statistically. The lack of knowledge concerning eosinophils is, of course, due to the difficulty of obtaining enough cells for adequate experiments. Recently we had the good fortune to see 2 patients who supplied us with great numbers of polymorphonuclear eosinophils. One of them had eosinophilic myelogenous leukemia and the other dermatitis herpetiformis. The chemotaxis of eosinophils obtained from these patients was studied in vitro with bacteria, *Trichinella spiralis* and Witte's peptone as sources of attraction. The chemotropism shown was compared with that of polymorphonuclear neutrophils obtained from healthy persons.

## METHODS

The eosinophils used in the experiments were obtained from two sources (1) the fluid of fresh bullae of the patient with dermatitis herpetiformis, (2) blood obtained by puncture of a finger of the patient with myelogenous leukemia of eosinophilic type. Blood obtained in the same manner from healthy adults was used as a source of neutrophils. The fluid of the bullae contained 76 per cent eosinophils, many of which were degenerate and moved poorly. The patient with leukemia had a total leukocyte count of approximately 30,000, with 70 to 80 per cent adult eosinophils, which morphologically were identical with the eosinophils seen in the circulating blood of healthy adults. The attracting substances used were (1) twenty-four hour cultures of *Staphylococcus aureus*, (2) dried, ground *Trichinella spiralis*, (3) Witte's peptone. The peptone was adsorbed to titanium dioxide in order to have a visible target.

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From the Institute of Pathology, Western Reserve University and University Hospitals

1 Rosegger, H. *Ztschr f d ges exper Med* 85 712, 1932

A fragment of one of the attracting substances was fixed on a glass slide by drying. A drop of blood or of fluid from a bulla was placed on a cover slip and spread over the test substance, which thus formed a small target on the slide. The movements of leukocytes near the target were observed under the microscope, and the course of each cell was recorded with the aid of a drawing ocular at one minute intervals for a period of ten minutes (fig 1). All the observations were made in a warm chamber at 37 C.

In these experiments the measure of chemotropism was defined as the net approach in microns per minute of each cell toward the test substance. In each group of experiments the results were expressed as the mean value of chemotropism of all the cells observed. The random movement of cells in fields remote from the target was observed in each preparation in order to make certain that directional currents were not present (fig 2). In the case of Witte's peptone, control experiments were made using plain titanium dioxide<sup>2</sup>. In order to set up a standard of comparison, polymorphonuclear neutrophils obtained from the finger blood of healthy adults were tested with the same substances as the eosinophils.

## RESULTS

The first series of experiments was designed to test whether eosinophils are attracted by two common bacteria, namely, *Staph aureus* and *Escherichia coli*. The results are shown in table 1. The mean value of chemotropism for 35 eosinophils from the blood of the patient with leukemia, tested against *Staph aureus*, was +9.0, with a standard error of  $\pm 1.51$ , whereas the value of chemotropism for 67 neutrophils from healthy adults was +7.5, with a standard error of  $\pm 1.20$ . The difference of the means with its standard error was  $1.5 \pm 1.94$ . Since the difference of the means is not more than twice its standard error these results are not significantly different from each other. In other words, eosinophils from the patient with leukemia were attracted as strongly as neutrophils from presumably healthy persons.

The eosinophils obtained from the fluid of the bullae were attracted less strongly by *Staph aureus* than were normal neutrophils. Although this difference is statistically significant, it is probably of little real significance because of the fact that these eosinophils were not healthy cells but showed obvious evidences of degeneration and poor locomotion. In the second column of table 1 it is seen that eosinophils from the bullae were attracted by *Esch coli*.

In the second series of experiments the effects of two substances known to produce eosinophilia in experimental animals were tested. The results are shown in table 2. Fifty-four neutrophils from healthy adults, tested with dried *T spiralis*, gave a mean value of chemotropism of +7.0  $\pm 0.33$ , and 224 eosinophils from the blood of the patient with leukemia

<sup>2</sup> Titanium dioxide has previously been shown to be chemotactically inert (unpublished data).



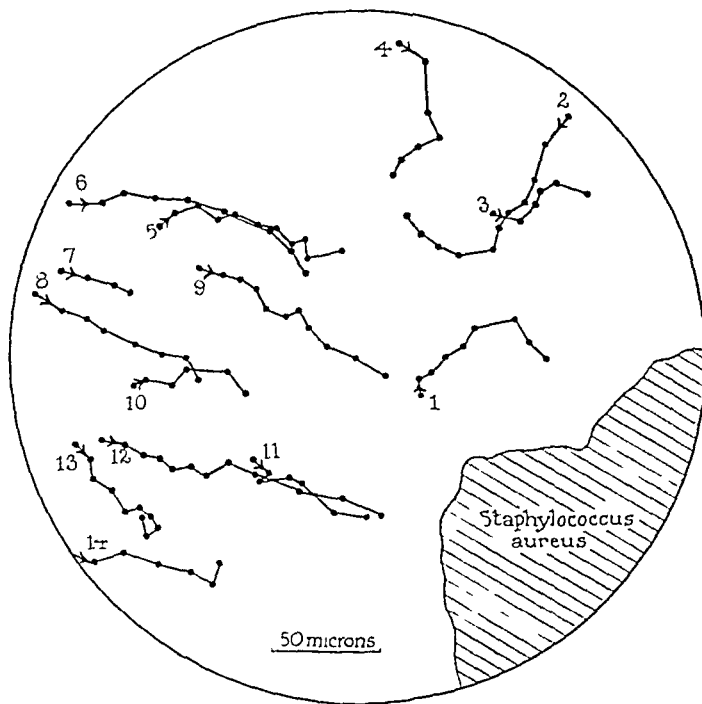


Fig 1—A camera lucida record of the attraction of human eosinophilic polymorphonuclear leukocytes to *Staphylococcus aureus*. The cells were obtained from the blood of a patient with myelogenous leukemia of eosinophilic type. The numbers represent the positions of the eosinophils when first observed, and the dots show successive positions at one minute intervals. The cells are moving toward the bacteria.

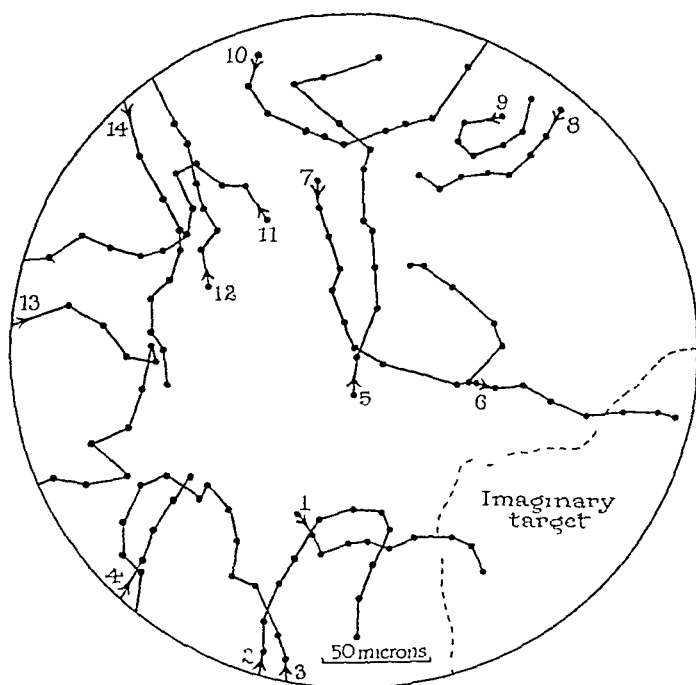


Fig 2—A camera lucida record showing random motion of eosinophils. This is the control of the experiment shown in figure 1. The imaginary target is obtained by projecting the outline of the bacterial target on the microscopic field. The cells show no directional movement.

gave a mean value of  $+5.8 \pm 0.99$ . The difference of the means and the standard error is  $1.2 \pm 1.06$ . Consequently there was no significant difference in the chemotactic behavior of the two types of cells. Similar results were obtained when Witte's peptone on titanium dioxide was employed as the test substance.

## COMMENT

These experiments indicate that in vitro eosinophilic polymorphonuclear leukocytes show the same degree of positive chemotropism as do

TABLE 1—*Chemotropic Response of Polymorphonuclear Eosinophils to Bacteria*

Polymorphonuclear Leukocytes Used	Staphylococcus Aureus		Escherichia Coli	
	Cells	Mean Value of Chemotropism in Microns per Minute with the Standard Error	Cells	Mean Value of Chemotropism in Microns per Minute with the Standard Error
Neutrophils from blood of healthy adults	67	$+7.5 \pm 1.20$	52	$+5.8 \pm 0.50$
Eosinophils from blood of patient with leukemia	35	$+9.0 \pm 1.51$		
Eosinophils from bulla fluid of patient with dermatitis herpetiformis	92	$+4.6 \pm 0.86$	63	$-4.7 \pm 1.20$

TABLE 2—*Chemotropic Response of Polymorphonuclear Eosinophils to Trichinella Spiralis and Witte's Peptone*

Polymorphonuclear Leukocytes Used	Dried, Ground Trichinella Spiralis		Witte's Peptone on Titanium Dioxide	
	Cells	Mean Value of Chemotropism in Microns per Minute with the Standard Error	Cells	Mean Value of Chemotropism in Microns per Minute with the Standard Error
Neutrophils from blood of healthy man	54	$+7.0 \pm 0.33$	16	$+9.8 \pm 1.04$
Eosinophils from blood of patient with leukemia	224	$+5.8 \pm 0.99$	75	$+8.6 \pm 0.02$

neutrophils. It is also interesting to observe that eosinophils are not attracted more strongly by *T. spiralis* than by bacteria, in fact they are attracted less strongly by this parasite than by Witte's peptone. Although the leukocytes employed are not strictly comparable to the eosinophils seen in normal human blood or in an inflamed area nevertheless they are probably similar cells. It is well known that polymorphonuclear eosinophils appear in both acute and chronic inflammations, but the manner in which they get to the inflamed tissues is not clear. Some believe that the eosinophils originate within the tissues either from previously existing cells or as derivatives of cells which have infiltrated from the blood. However, since it has been shown that eosinophilic

polymorphonuclear leukocytes possess positive chemotropism, it seems at least possible that they may be actively attracted to a site of inflammation in the same fashion as the polymorphonuclear neutrophils

Eosinophilic polymorphonuclear leukocytes are also capable of phagocytosis. This has been shown by several workers,<sup>3</sup> and we have been able to confirm their observations. Thus it is seen that the eosinophil behaves much like the neutrophil in that it shows both phagocytosis and chemotropism. In contrast to these two cells is the lymphocyte which, so far as is known, shows neither phagocytosis<sup>3a</sup> nor chemotropism.<sup>4</sup>

#### SUMMARY

The chemotropism of human polymorphonuclear eosinophils was studied *in vitro*. The cells were obtained from a patient with eosinophilic myelogenous leukemia and from one with dermatitis herpetiformis. The chemotactic reaction of eosinophils to bacteria was found to be as strong as that of normal neutrophilic leukocytes. Eosinophils were attracted less strongly by material from an animal parasite, *T. spiralis*, than by Witte's peptone. The origin of eosinophils in the tissues is discussed.

Dr. Joseph T. Wearn gave us permission to study the 2 patients mentioned in this article.

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3 (a) Strumia, M. A., and Boerner, F. *Am. J. Path.* **13** 335, 1937. (b) McDonald, S., and Shaw, A. F. B. *Brit. M. J.* **2** 966, 1922.

4 Dixon, H. M., and McCutcheon, M. *Arch. Path.* **19** 679, 1935.

# GENESIS OF INFARCTION

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DECATUR, ILL

The genesis of infarction in the human body is not as yet clearly understood. The question still remains why in one organ—the lungs or intestines, for example—the infarct is practically a hemorrhagic one, while in other organs—the kidneys or spleen—an anemic infarct is quite as regularly found. It is difficult to explain why stoppage of the blood supply to one organ may produce hemorrhagic necrosis, while stoppage of the supply to another produces anemic necrosis in spite of collaterals present.

Previous experiments reported in this journal<sup>1</sup> showed that ligation of the mesenteric artery in rats produces anemic necrosis, while ligation of the portal veins produces a lesion resembling hemorrhagic infarction.

Because the animals lived only for hours after the ligation of those large vessels, it was felt necessary to continue the experiments.

The following experiments on intestinal infarction may be reported because of their simplicity and unequivocal results and because the tissue changes are much more distinct than those in previous experiments. The results seem to be well comparable to findings in the human body.

## PROCEDURES

Altogether 60 animals, including 12 guinea pigs and a few rabbits, but mostly white rats, have been used.

Sodium amytal and ether were the anesthetics used, the latter was found to be more convenient.

*First Series (10 Rats and 6 Guinea Pigs)*—A loop of small intestine about 4 to 6 cm in length, together with its mesentery, was firmly tied with a thick thread. The tying was done as quickly as possible, within a fraction of a second. After a few minutes the loop contracted both in length and in width. The little blood of the loop drained into the mesenteric veins up to the tying point. Only the smallest intramural or serosal veins of the loop remained visible, these were, however, greatly narrowed.

After three to six hours the loop began to look dull, gray and flabby. There was no change in the lumen.

The next day the loop was as white as pale pus, entirely without tonus and dull in appearance, this was even more pronounced forty-eight to seventy-two hours after the operation. The lumen was narrow and empty (fig 1A).

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From the Pathological Laboratory of the Decatur and Macon County Hospital  
1 Loeffler, L. Arch Path **22** 755, 1936

All animals died after one to three days from intestinal obstruction. The loops above the obstruction were found dilated and reddened. There was a thin purulent fibrinous membrane around the tied loops and adjacent tissue, mostly omentum, however, there was no general peritonitis.

Microscopically, the signs of necrosis were obvious after twenty-four hours, beginning with loss of structure and falling apart of cells (fig 2 *A*). This was

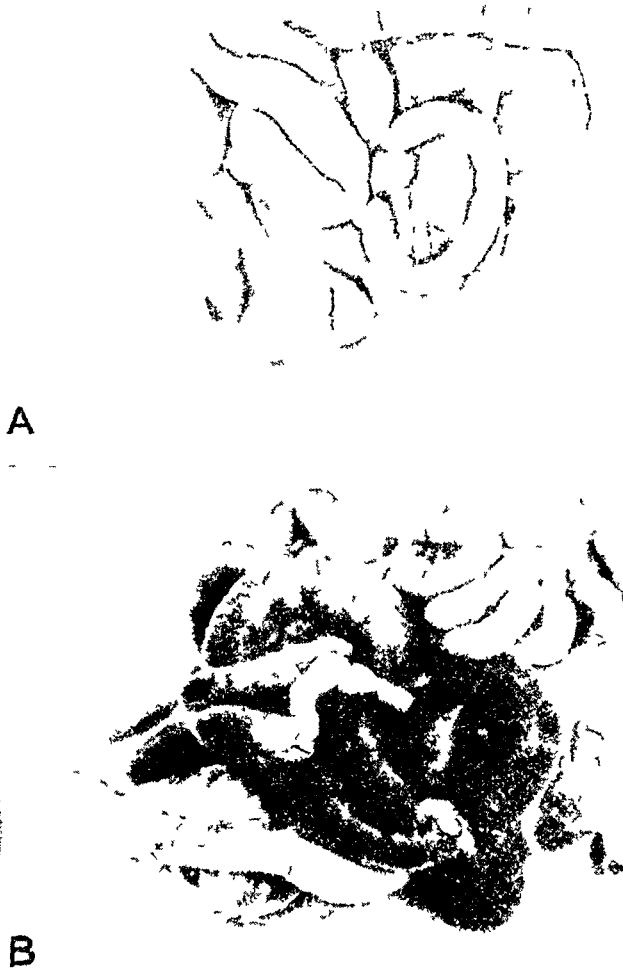


Fig 1—*A*, anemic necrosis of an intestinal loop of a guinea pig one day after ligation. The ligature was suddenly and completely tied. About actual size. *B*, hemorrhagic infarction of an intestinal loop in a guinea pig one day after ligation. The ligature was slowly and incompletely tied. About actual size.

followed by loss of nuclear staining of the mucosa, the musculature retaining its staining power until death. Here and there small serosal veins could be found filled with well preserved erythrocytes. The serosa was smooth or covered with fibrin and leukocytes (fig 2 *B*).

*Second Series (10 Rats and 6 Guinea Pigs)*—An artificial volvulus was produced. An intestinal loop was held between two fingers, rolled around itself in a spiral fashion and loosely fixed in that position by a string at the base and a suture

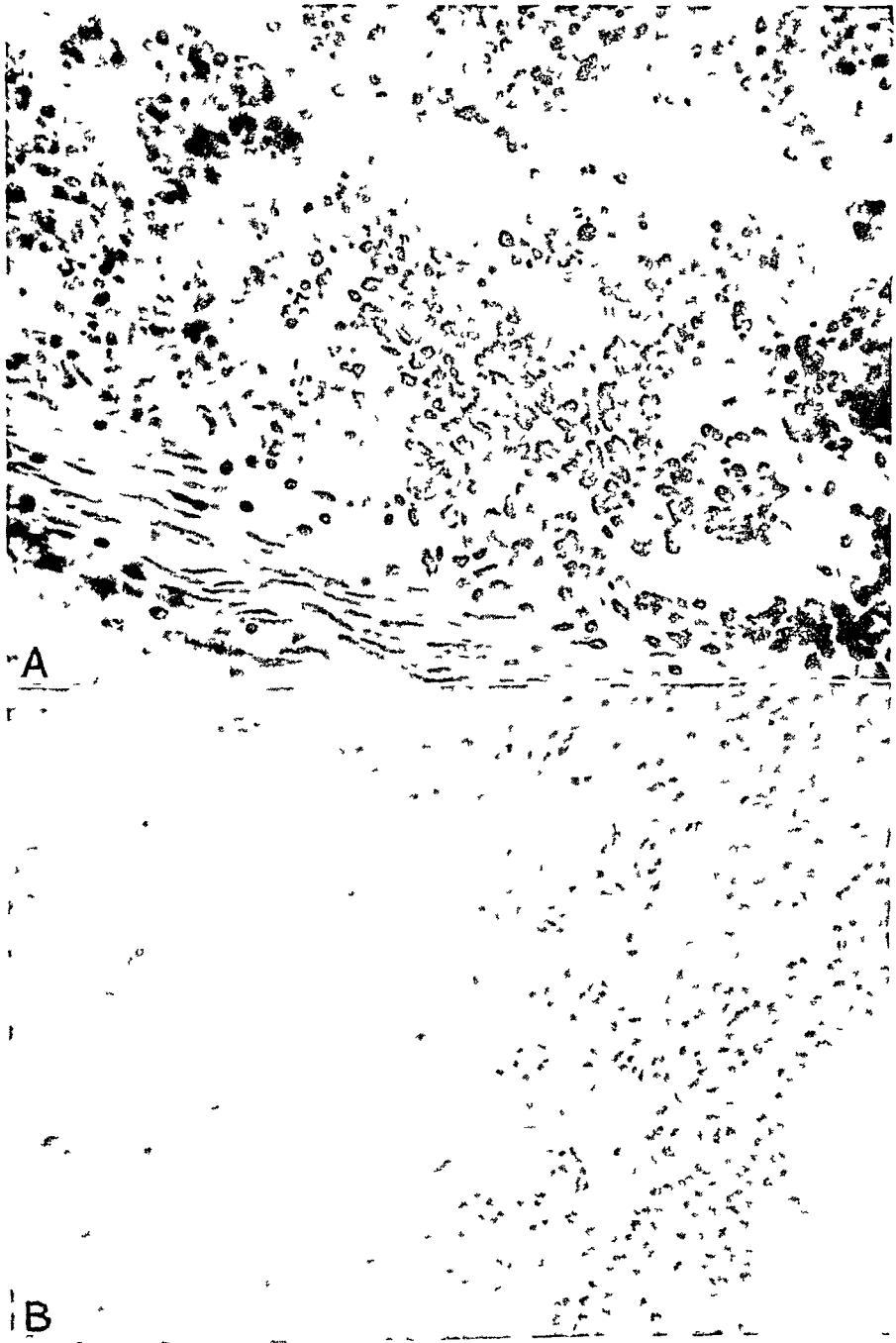


Fig 2—*A*, beginning anemic necrosis one day after ligation. *B*, complete necrosis three days after ligation,  $\times 200$ . A layer of leukocytes and fibrin is seen at the right in *B*.

through the serosa. In the guinea pigs it was only necessary to set two ligatures with a thick thread, the first near the base of the mesentery and the second near the intestinal wall about 1 to 2 cm. distant from the first.

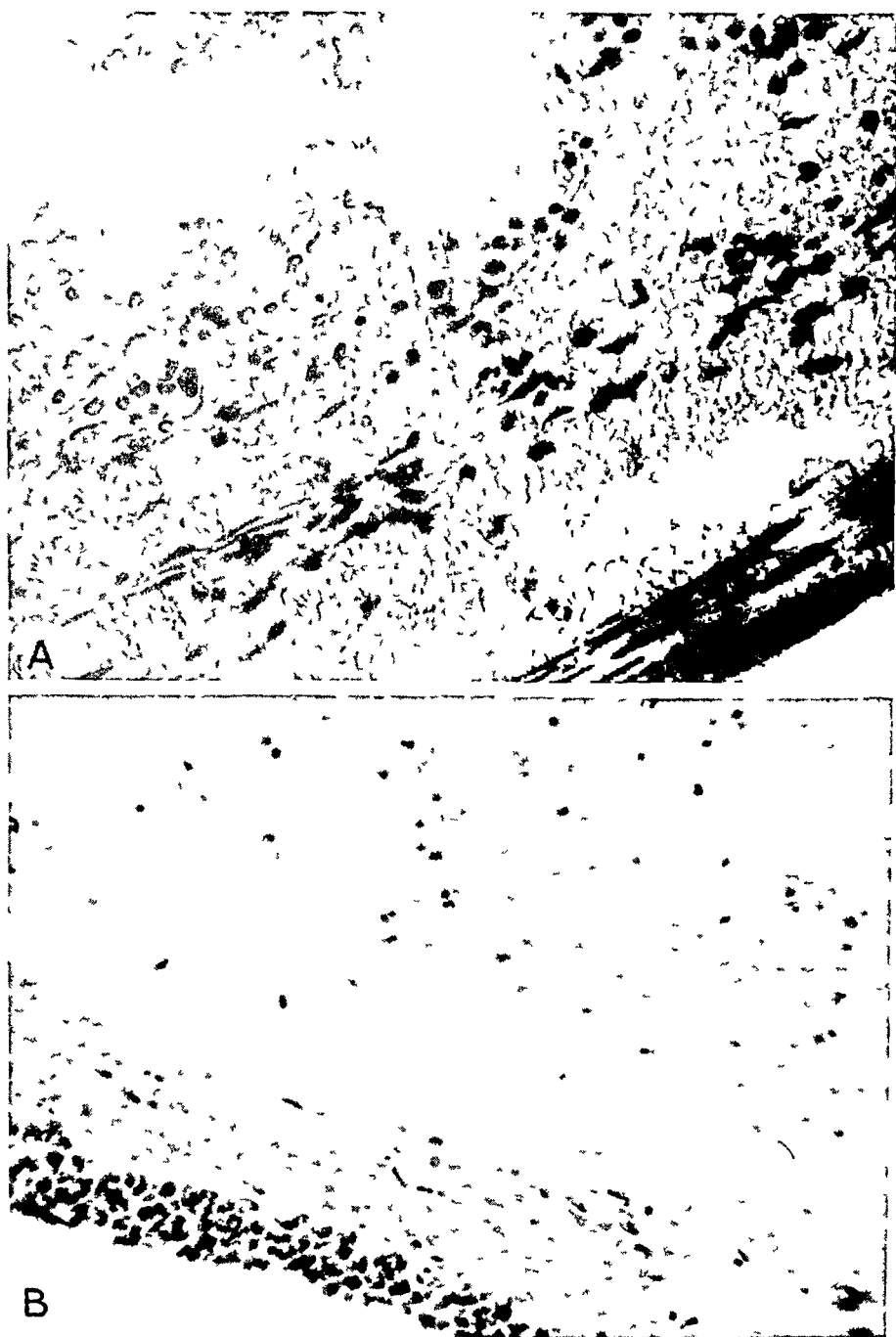


Fig 3—*A*, hemorrhagic infarction of an intestinal loop one day after ligation,  $\times 200$ . Masses of erythrocytes are seen within the broken-up wall. *B*, hemorrhagic infarction three days after ligation,  $\times 200$ . Necrosis is completed. The shape of the erythrocytes is at places still seen. A layer of leukocytes at the bottom of the picture is well stained in sharp contrast to the necrosed wall.

It was necessary to observe the circulation in the mesentery and see that all ligatures were only narrowing and not entirely obstructive of the blood supply. Pulsation in the dilated vessels was noticed where the mesentery bordered the intestinal wall.

Extreme care, however, was not necessary, there was no failure in any of the experiments—quite an unusual feature in experimental work. Each experiment was finished in from fifteen to twenty minutes.

After twenty-four hours the intestinal loop on top of the volvulus, most often both loops, were greatly dilated, dark black-red, hemorrhagic. The loops were filled with turbid bloody exudate. The serosa was dull (fig 1 *B*).

In some cases there was local peritonitis around the loop with fibrinous adhesions after two or three days but no general peritonitis.

All animals died after three days from intestinal obstruction, as did those of the first series.

Microscopically, the typical picture of hemorrhagic infarction was seen. All vessels were greatly dilated and filled with erythrocytes, and there was complete destruction of the mucosa and submucosa by masses of extravasated erythrocytes (fig 3 *A*). Nuclei ceased to stain after two or three days, pyknotic and broken-down nuclei were found in abundance. The musculature was less hemorrhagic than the inner layers and retained its staining power until death. The intestinal wall was thinned (fig 3 *B*).

#### COMMENT

In the first series of experiments an intestinal loop was shut off suddenly and completely from its blood supply. The result was without exception anemic necrosis of the intestinal loop. This is what one would expect from such an operation, an organ deprived of its blood must be anemic and must undergo necrosis in due time. It is understood that a short time after the ligature has been set blood is still in the vessels giving the tissue a slightly red color. The red changes into white with advancing destruction and dissolution of tissue and erythrocytes. After one to two days not a trace of red remains in any one of the loops.

The observed contraction of the muscular tissue of the wall is secondary to the anemia. Contraction sets in after a few minutes, driving the remaining blood into the mesenteric veins. The muscular contraction, however, cannot be held entirely responsible for the anemia observed. It is the anoxemia which produces the contraction. The musculature finally loses its tonus, the wall becomes flabby. The mucosa disintegrates first. Then the cells of the mucosal glands fall apart and gradually lose their nuclear staining within one to two days. The nuclei undergo karyorrhexis and pyknosis.

A secondary factor influencing the staining properties of the involved tissue is the intestinal juices, the intestinal juices must be taken into consideration, for they might loosen the structures and accelerate the disintegration, however, their influence is easily recognized by the uniformity of decolorization and the depth in diffusion, ending in a sharp line without a reactive zone. The picture here does not show such changes, therefore this factor may be disregarded.



A layer of fibrin and leukocytes is in some instances formed on the necrosed loop. It must be assumed that fibrin and leukocytes come from adjacent tissues and are deposited on the serosa, since there is no circulating blood available to act as a source for these blood constituents.

The ligature in the second series was not tied completely and rapidly as in the first but was incompletely and slowly tied so as to permit a low and diminished circulation of blood. The first effect of such a procedure is venous congestion lasting for hours, with visible pulsation of arteries and veins. Dilatation of the intestinal wall follows. To this dilatation and the twisting of the loops must be attributed the final stoppage of the circulation. The twisted loop is black-red, dull, greatly dilated and filled with bloody fluid mixed with fecal material. The loop stays that way to become enveloped by adjacent loops and omentum until death. Death occurs not later than three days after operation.

The gross picture leaves no doubt that a truly hemorrhagic infarction took place. The microscopic picture is not that of mere congestion with occasional petechiae but that of a uniform heavy hemorrhage into the intestinal wall, identical with that found in the human body in similar cases.

In the experiments recorded here complete and sudden stoppage of the blood supply to the intestinal wall caused anemic necrosis in all the animals without exception. Therefore, there is not an inherent, unexplained and unexplainable tendency of the intestines toward hemorrhagic infarction. The hemorrhagic character of the lesion depends on the special conditions. If the stoppage is sudden and complete, anemic necrosis results, if it is not sudden and complete, hemorrhagic necrosis may result. Now sudden and complete stoppage is practically never realized in a human body. No hernia is incarcerated in a second or at least not completely. It takes time, and half an hour is probably enough to cause venous congestion which slowly aggravates to stasis and infarction. The same is true in cases of volvulus or intussusception. Whether the blood comes from still open vessels or from nearby collaterals can, of course, make no difference. The important factor is the completeness of stoppage of blood supply to the intestinal wall.

The consequences of vascular occlusion on the bowel vary. The superior mesenteric artery may be occluded by thrombosis or embolism without any changes of the intestines (cases have been reported by Dye<sup>2</sup>, McIver<sup>3</sup>, Ross<sup>4</sup> and earlier yet by Virchow<sup>5</sup> and Karcher<sup>6</sup>).

2 Dye, W. J. P. *New England J. Med.* **212** 105, 1935.

3 McIver, M. A. *Am. J. Surg.* **20** 195, 1933.

4 Ross, G. G. *Ann. Surg.* **72** 121, 1920.

5 Virchow, R. *Arch. f. path. Anat.* **1** 272, 1847, cited by Warren, S. and Eberhard, T. P. *Surg., Gynec. & Obst.* **61** 102, 1935.

6 Karcher, J. *Cor.-Bl. f. Schweiz. Aerzte* **27** 548, 1897, cited by Dye<sup>2</sup>.

Often at autopsy on persons dying from other causes an embolus is found in this artery without any changes of the bowel. In other cases (2 per cent, according to Trotter<sup>7</sup>) anemic gangrene is found. Still in other cases the changes of the intestine vary from congestion, edema and other reactions to complete infarction and necrosis (McIver<sup>8</sup>).

In 23 per cent of cases Whittaker and Pemberton<sup>9</sup> found arterial occlusion combined with venous thrombosis. The occurrence of venous thrombosis is probably higher than that of pure arterial thrombosis (Warren<sup>10</sup>). The observations in cases of anemic necrosis following arterial occlusion compare closely with these experimental findings.

The cases in which there are no changes are generally explained by collateral vessels.

Cases of arterial occlusion combined with venous thrombosis and with hemorrhagic infarction correspond to the experiments with slow and incomplete occlusion.

The rest, namely, cases of pure arterial occlusion with infarction but with patent veins and collaterals, are still unexplainable.

This much, however, can be drawn from these experiments: 1. Complete and sudden stoppage of the blood supply to the intestines (and probably to any organ) causes anemic necrosis. Hemorrhagic infarction is due to incomplete stoppage. Blood must be allowed afterward to enter the area affected, it makes no difference where this blood comes from—from collaterals, veins or arteries. 2. This blood must enter and remain under sufficiently high pressure. This is realized in cases of venous thrombosis following arterial embolism or in cases of volvulus by venous congestion. Similar conditions exist in cases of incarcerated hernia or intussusception.

The question asked in the introduction as to why in one organ—the lungs or the intestines, for example—a hemorrhagic infarct is usually found, while in other organs—the kidney or spleen—an anemic infarct is regularly found, might now be answered in this way. It is not true that organs differ from one another under the same conditions but it is true that different organs respond to the same conditions in the same way. In the intestines it has been proved that a complete and sudden stoppage of the blood supply leads to anemic necrosis, the same is true in the kidneys. If in the intestine a hemorrhagic infarct is practically always found, it must be due to special conditions, i. e. the stoppage of the blood supply must be practically never complete and sudden, and there must be pathways left open allowing blood to enter through arteries, veins or collaterals.

7 Trotter, L. B. C. *Embolism and Thrombosis of Mesenteric Vessels*, London, Cambridge University Press, 1913, cited by McIver<sup>8</sup>.

8 McIver,<sup>3</sup> p. 196.

9 Whittaker, L. D., and Pemberton J. de J. *J. A. M. A.* **111** 21, 1938.

10 Warren, S., and Eberhard, T. P. *Surg. Gynec. & Obst.* **61** 102, 1935.

## SUMMARY

The complete and sudden stoppage of the blood supply to the intestines (and probably to any organ) results in anemic necrosis. A hemorrhagic infarction is due to a special condition. Blood must enter the affected area after the initial closure, this blood must come to stay under high pressure. This is realized in cases of combined arterial and venous occlusion, of incarcerated hernia, of volvulus, of intussusception. In none of these cases is there a sudden and complete stoppage of the circulation.

Where the blood in hemorrhagic infarcts comes from seems to be of minor importance. It may come from patent veins or arteries or from collaterals.

It is postulated that the conditions leading to anemic or to hemorrhagic necrosis are the same in any organ.

# INCIDENCE AND SIGNIFICANCE OF HEALED MILIARY TUBERCLES IN THE LIVER, SPLEEN AND KIDNEYS

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The occurrence of small calcified spheres in the liver, spleen and occasionally other of the parenchymatous viscera is well known to pathologists and roentgenologists. Little attention, however, has been given to these lesions in the current medical literature. From time to time they have been referred to as "healed hematogenous tubercles," but more commonly they have been called "phleboliths."<sup>1</sup>

Because of a tentative hypothesis that these bodies are miliary tubercles, a systematic study was conducted on material obtained at autopsy in 500 consecutive cases at the City Hospital, Cleveland, from August 1935 to April 1936. In the search for these lesions, particular care was taken to examine the organs for manifestations of active, latent or healed tuberculosis. Approximately two thirds of the lungs were injected with solution of formaldehyde U. S. P. (diluted 1 to 10) and sectioned. The remaining materials were examined in the fresh state. In a few cases examinations were made with the aid of a fluoroscope, but in the majority the usual methods of palpation and section were depended on to reveal one or more of the components of a primary tuberculous complex, and if they failed to do so the results were recorded as negative.

For purposes of a statistical analysis, partial autopsies and autopsies on stillborn infants, premature infants and infants who died before discharge from the hospital were excluded. There were 48 such cases, leaving a remainder of 452. Nodules were found in one or several of the parenchymatous organs in 91 cases, an incidence of 20.1 per cent for the series. This is a high incidence, since few conditions other than parenchymatous degeneration, hyperemia, edema, primary tuberculosis and arteriosclerosis occur in more than one fifth of the bodies coming to autopsy in this hospital.

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1 Moorman, L. J. *Am. Rev. Tuberc.* **36**: 376, 1937. Berman, T. M. *Radiology* **29**: 37, 1937. Hellgren, E. G. *Acta pædiat.* **13**: 180, 1932.

The spleen was most commonly affected. The lesions were found in the spleen in 73 of the 91 cases, in the liver in 47 and in the kidneys in 2. In the liver they were usually situated just beneath but not within the capsule and were only occasionally found in the deeper parenchyma. In the spleen, on the other hand, they were commonly encountered deep in the pulp and infrequently beneath the capsule. The nodules bore no necessary relation to the structure of the organ in which they occurred or to its blood vessels. They could be easily extracted from the parenchyma but could never be shelled out of a casing such as is consistently found about a phlebolith. The number of lesions varied widely from case to case. In a few only one or two were found while in others they numbered several hundred.

The nodules were spherical and varied from 1 to 5 mm in diameter, with the majority being approximately 2 mm. Occasionally adjacent lesions had coalesced to form lobulated masses, but more commonly they were discrete and widely separated from their neighbors. Grossly they were hard, varied in color from pale yellow to gray and cut with gritty, sometimes bony resistance. When sectioned, they were found to consist of a soft, receding, pale yellow or gray homogeneous center and a thick, pale yellowish gray shell-like periphery. Microscopically (fig. 1) the center was composed of necrotic debris and sometimes a fine fibrillary network with scattered fibroblasts, large mononuclear cells and lymphocytes. In some the center was entirely fibrous. In about half of the nodules the caseous center was surrounded by a ring of hyalinized tissue containing collections of epithelioid cells, which formed radial palisades, lymphocytes and very occasionally a shrunken multinucleated cell. The periphery of the lesion consisted of a thick, extremely well circumscribed capsule of dense collagenous connective tissue which did not contain elastic fibers and which was similar to the specific capsule described by Puhl for the primary tubercle. Very few of the cell nuclei of connective tissue remained, and wandering cells, if present at all, were found only in the outer, looser layers. Varying quantities of calcium were present in the centers or capsules and in some bone formation had occurred.

Both grossly and microscopically the lesions were indistinguishable from small primary tubercles, i. e., Ghon foci, or from the healed satellite tubercles so frequently encountered in the lungs. They differed from true phleboliths in regard to both position and character and were sufficiently different from small adenomas, fibromas and adrenal rests to prevent confusion with these abnormalities. Because of their gross and microscopic similarity to lesions adequately established as healed tubercles, the assumption that they were monuments to previous tuberculous inflammation appeared justified. Furthermore an analysis of the data indicates a high positive correlation between the presence

of occult tuberculosis, either healed or active, and the occurrence of calcified nodules in the abdominal viscera. In 86 of the 91 cases (94.5 per cent) in which these nodules were found there was definite healed or active tuberculosis in some other part of the body. In only 5 cases were no tuberculous lesions found and in only 1 of these were roentgen studies undertaken. Further convincing evidence was afforded by the demonstration of tubercle bacilli in these lesions by animal inoculation. Twenty guinea pigs were inoculated with material from the lesions in

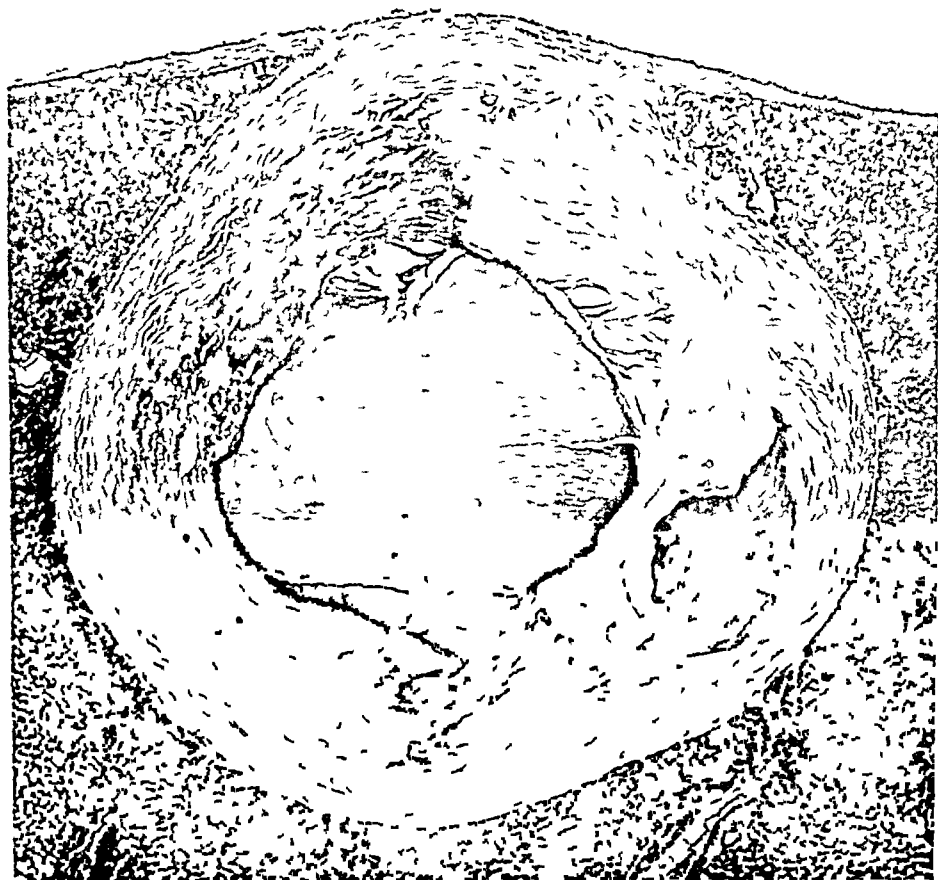


Fig. 1—Tubercle in liver, hematoxylin-eosin,  $\times 375$

14 cases. Precautions were taken to prevent contamination of the material, and no lesion was used if it was associated with active milary tuberculosis. Because of their small size it was not possible, however, to divide the lesions and use one-half for microscopic study. Positive "takes" were obtained in 4 animals in 3 of the cases.

The morphologic similarity of these lesions to those of healed primary tuberculosis, their concurrence with admittedly tuberculous lesions and the evidence that a fair number of them harbored tubercle bacilli establish them as healed or obsolete tubercles. Their distribution in liver,

spleen and kidneys was such as to indicate that they were the result of hematogenous dissemination. This consideration leads to the following questions:

1. Does hematogenous dissemination have any relation to the development of primary tuberculosis?

2. What effect, if any, does it have on the patient?

It is well known that in large communities most persons who acquire a primary infection do so before the age of 30 years. Therefore, the incidence of these tubercles should be much the same in all age groups over 30 years of age if dissemination occurs early in the course of a primary infection. If, on the other hand, it is a protracted process, occurring from time to time during the lifetime of an infected person, the incidence of healed lesions should bear a direct relation to age. That this is not the case is well shown by figure 4. These lesions therefore developed at a time when the state of reaction characteristic of the primary phase of infection was still active. This constitutes morphologic evidence which demonstrates the probable reality of the second stage in Ranke's classification of the pathogenesis of tuberculosis. Hitherto this stage was regarded as largely hypothetical and as merely bridging the gap between the primary and tertiary stages. In contradistinction to Ranke's opinion, we do not regard stages I and II as sequential, except for the earliest period, which is a matter of days rather than weeks, the two stages run parallel to one another in time. The generalization starts before the primary focus has developed, but the disseminated bacilli, being subject to inherent immunity as well as to the awakening acquired resistance, are reduced in number. The incubation time of primary foci, however, is dependent in a large measure on the number of bacilli. Hence these disseminated foci will develop somewhat later than the primary focus itself.

It is generally accepted that hematogenous dissemination is a common accompaniment of progressive primary tuberculosis and that many of these patients, particularly young children and members of the colored race, die of tuberculous meningitis and generalized miliary tuberculosis. However, there is also abundant clinical, pathologic and experimental evidence that hematogenous dissemination occurs in persons who do not die of the primary infection. One of the cases in our series and another observed subsequently illustrate very well that dissemination can occur early during the course of primary infection. In the first instance a white boy of 10 years died as the result of severe acute streptococcic pansinusitis, bronchitis and mild leptomeningitis, following scarlet fever. At autopsy a typical primary complex was found in the upper lobe of the right lung and in the bronchopulmonary and superior and inferior bifurcation lymph nodes. All parts of the complex showed an exudative type of reaction, and there was no gross caseation of the central por-

tions In addition, a few active miliary tubercles were demonstrated in the spleen In the light of previous experience the process was regarded as not older than three months Nevertheless, there were well developed tubercles in the liver and spleen In the other case a Negro child of 15 months had been given a clinical diagnosis of tuberculous meningitis, pulmonary tuberculosis and generalized miliary tuberculosis This child had been exposed to tuberculous disease in the father, who was admitted to the Lowman Pavilion of the City Hospital when the child was only 3 months old There were no other contacts The primary focus was located in the upper lobe of the left lung It had undergone central liquefaction and cavitation and was surrounded by a thick fibrous wall The lymph nodes were unusually large and caseated There were a tuberculoma of the brain, tuberculous meningitis and widespread active hematogenous tuberculosis Of considerable interest was the presence of one perfectly typical healed tubercle in the spleen in addition to active tubercles The former was calcified and when examined microscopically showed no sign of activity The observations indicate partial control of a primary infection with subsequent reactivation, widespread dissemination and death The difference in age of the splenic lesions points to an early as well as a late dissemination The chronic nature of the pulmonary disease, as well as the healed tubercle in the spleen, clearly indicates that this child must have had tuberculosis for a considerable period and the history establishes the fact that the primary infection occurred some time during the first three months of life It takes weeks for a primary lesion to develop and months or years for lesions even as small as this to heal and calcify Therefore the early dissemination presumably occurred between the second and third months of life or within six weeks or two months after the primary infection These 2 cases conclusively show that dissemination can and does occur very early in the course of the primary disease This is no new concept of the pathogenesis of human tuberculosis,<sup>2</sup> and it has been shown that bacillema can exist in experimental animals long before the appearance of a primary lesion Krause<sup>3</sup> showed that tubercle bacilli may invade the lymphatic and the vascular system within four days of inoculation and become disseminated throughout the body Willis<sup>4</sup> continued these experiments and came to the conclusion that in guinea pigs inoculated via the skin visceral tuberculosis usually develops even though the area of inoculation is excised within three hours after inoculation In this respect infection with the tubercle bacillus resembles that with *Spirochaeta pallida*<sup>5</sup>

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2 Grethmann, W Arch Path **23** 451, 1937

3 Krause, cited from Willis<sup>4</sup>

4 Willis, H S Am Rev Tuberc **11** 439, 1925

5 Raiziss, G W, and Severac, M Arch Dermat & Syph **35** 1101, 1937



The significance of this early hematogenous dissemination and of its effect on the infected person was investigated by studying the correlation between the incidence of these healed miliaary tubercles and the presence of active tuberculous disease. All cases in which lesions of tuberculosis were shown were divided into three groups according to the apparent resistance which was exhibited to the disease (fig 2). Into group 1 were placed all cases in which there was neither clinically significant disease nor a lesion showing only microscopically demonstrable activity. The bulk of these cases were those in which only one or more of the components of a primary complex were observed and

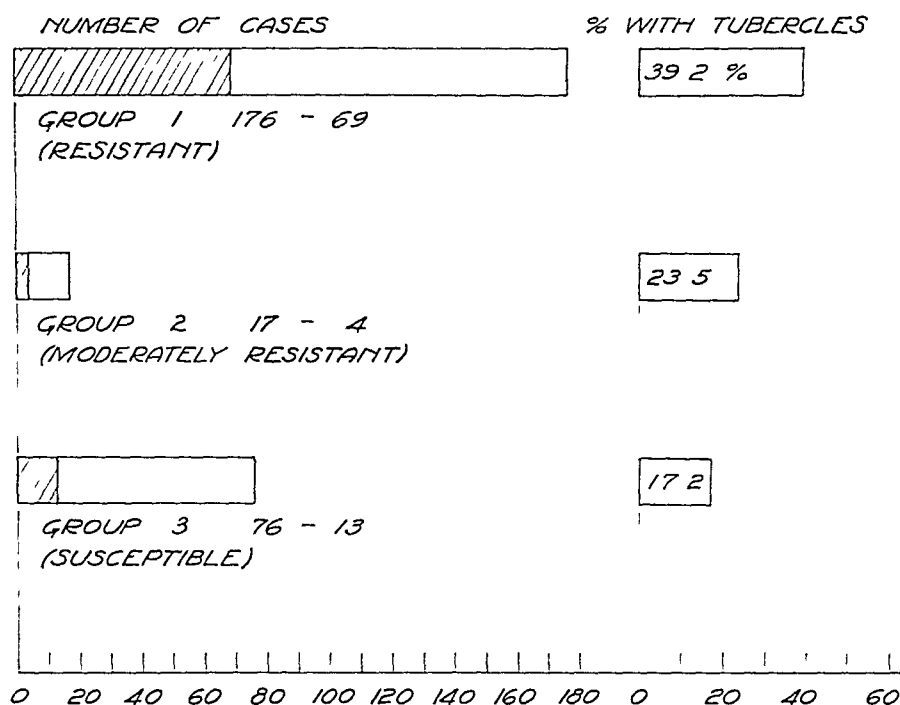


Fig 2—Analysis of all cases

the patients were designated as resistant. Group 2 included cases in which there was no clinically significant disease but in which microscopic sections showed activity, the patients were designated as moderately resistant. Group 3 included only cases in which there was clinically manifest, active disease. In the vast majority of these cases death occurred as a result of tuberculosis. The patients in this group were designated as susceptible. Figure 2 makes it apparent that the incidence of healed miliaary tubercles in the so-called susceptible group is only half of that in the resistant. Furthermore, the incidence in the moderately resistant is about midway between that in the resistant and that in the susceptible group. In figure 3 the white persons and the Negroes have been represented separately. Again the highest incidence is found

in the so-called resistant persons of each race and the lowest incidence is in the susceptible. The number of cases in group 2 is probably too small to be of any statistical significance, yet, as in figure 2, the percental values are intermediate between the other two groups. No adequate explanation for the differences between the two races is offered. Figure 4, referred to earlier in this paper, shows that there is no correlation between the chosen age groups and the incidence of healed military lesions in the spleen and liver. In spite of the small numbers in some of the groups, the differences already noted between the susceptible and resistant groups are still apparent.

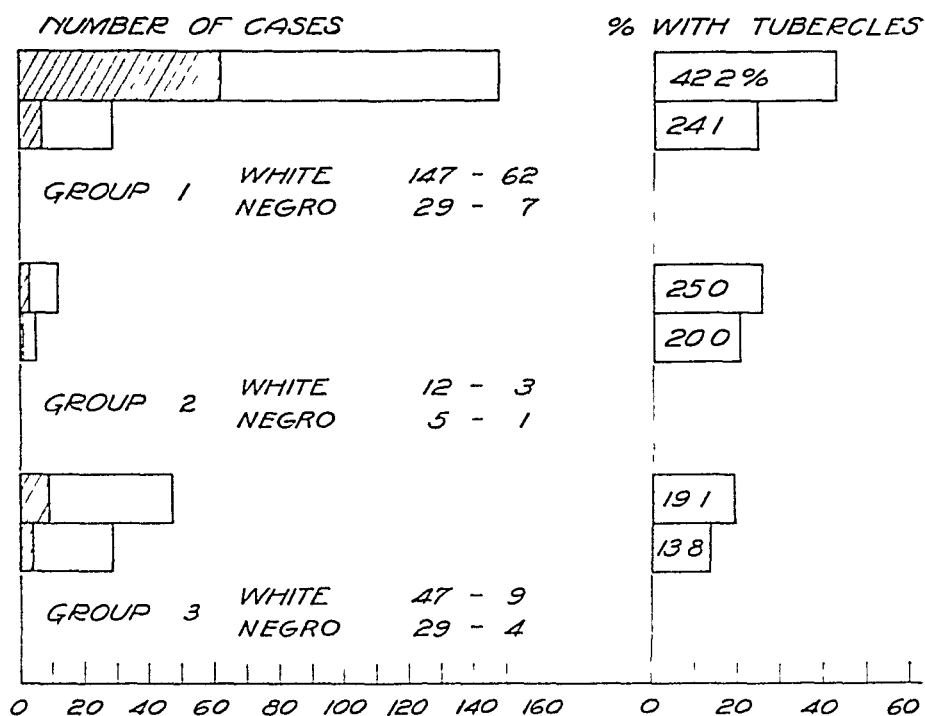


Fig 3—Analysis by race

From these diagrams several valid conclusions can be drawn. First, there is no correlation between age and incidence of healed military tubercles. Second, the percental incidence of healed tubercles is greater in the white than in the Negro race. Third, the incidence of healed tubercles is greater in persons who, on the basis of anatomic findings, have been considered resistant than it is in more susceptible persons, and these differences are constant for both races. For this there are at least two possible explanations. One is that a large number of the more susceptible succumb to the infection at the time of the early hematogenous spread. If this were the correct and only explanation, however, it might be expected that, if all persons dying of progressive primary tuberculosis, as well as persons dying from other causes with incidental

active primary lesions, were deducted from the total number of patients of group 3, the percental incidence of healed milary tubercles computed on the basis of the corrected figure would approximate that of the first group. There were only 2 such persons in group 3, and both of these were children who died of causes other than tuberculosis and presented at the time of autopsy active primary complexes as well as active milary tubercles in the spleen. With these 2 patients deducted from group 3 the incidence of healed tubercles is raised to only 18 per cent, a figure which is still significantly different from 39.2 per cent, the incidence in group 1. The second explanation for this difference is that an early hematogenous spread, with limited seeding of tubercles in the organs,

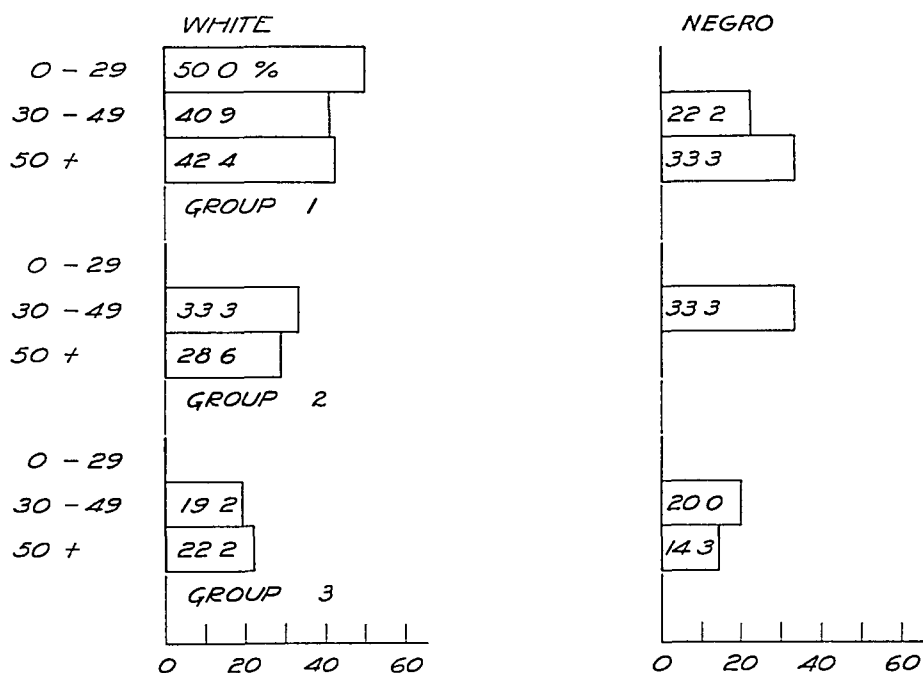


Fig. 4—Percental analysis of cases by race and age

acts as an "autovaccination" and confers protection against reinfection. That the spread of a disease should serve to check the progress of that disease at first appears to be a paradox. It is, however, well known that this does occur in tuberculosis. Persons with extensive pulmonary disease rarely die of milary tuberculosis, and, on the contrary, pathologists have long known that typical acute milary tuberculosis of the adult is usually associated with some relatively unimportant form of organ tuberculosis.

Had accurate counts of the number of tubercles present in the liver, spleen and other organs been made in each case, it is possible that the figures thus obtained would have served as rough indexes of the degree or intensity of the dissemination. And, if so, a positive correlation

between the number of tubercles and the manifest resistance might be expected. This aspect of the problem was not appreciated until it was too late to make such counts. A review of the lung-diagrams made at the time of autopsy, however, suggests that dissemination was generally more widespread in the relatively resistant than in the relatively susceptible. In 22 of the 69 cases in group 1 or 31.9 per cent, there were calcified tubercles in the lung parenchyma other than the primary focus. This compared with 1 of 4 (25 per cent) and 1 of 13 (7.7 per cent) in groups 2 and 3, respectively.

#### SUMMARY

Many, if not all, of the small spherical bodies found in the liver, spleen and kidney and frequently called phleboliths are true milary tubercles. They occur in approximately one fifth of the bodies coming to autopsy at the City Hospital, Cleveland. They are histologically indistinguishable from small primary tubercles. Animal inoculation has demonstrated the presence of tubercle bacilli. Their distribution points to hematogenous dissemination and their morphology to some association in point of time with the primary complex. The high correlation between apparent resistance to progressive tuberculous disease and the incidence of these lesions leads to the suggestion that this dissemination acts as autovaccination, conferring a relatively high resistance to reinfection.

# ACTION OF ESTROGEN ON SKELETAL TISSUES OF IMMATURE GUINEA PIGS

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In previous investigations<sup>1</sup> we found that gonadectomy had a growth-promoting effect on the euhyaline cartilage of the immature guinea pig. Differences in the response of the sexes were observed. The proliferation was somewhat more marked in male animals, in these, likewise, the balance between proliferation, on the one hand, and calcification and ossification, on the other, was more disturbed. In pursuance of these investigations we thought that a study of the action of estrogen might give further insight into the role of the gonads in the processes of growth and ossification of cartilage.

## MATERIAL AND METHODS

Forty-eight guinea pigs, born in the spring, were used, 24 of these animals were given an intraperitoneal injection of 20 rat units of estrogen dissolved in 0.9 per cent saline solution, six times weekly, 24 others were given each a dose of 250 rat units of estrogen in oil subcutaneously, twice weekly. In each of these two groups 12 animals were males and 12 were females, at the beginning of the experiments one half of the guinea pigs weighed between 140 and 160 Gm and the remaining half between 180 and 190 Gm. The large majority of the animals were killed three, seven, fourteen, twenty-one, thirty and sixty days after the injections were started, but 3 guinea pigs had to be killed for various reasons at intermediate times.

At autopsy, one tibia, the knee joint of the other side, ribs and vertebrae were removed for study and subjected to the same technical procedures as previously described.<sup>2</sup>

## CHANGES IN WEIGHT

The variations in the weights of the animals were similar in the different groups. Slight differences became apparent only in those

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1 Silberberg, M., and Silberberg, R. *Am J Path* **15** 55, 1939.

2 Silberberg, M., and Silberberg, R. *Arch Path* **26** 1208, 1938.

animals in which the injections of estrogen were continued for two months. In table 1 the initial and final weights of these animals are given the first two horizontal rows representing the weights of the guinea pigs which weighed about 150 Gm at the beginning of the experiment and the last two horizontal columns the corresponding figures for the animals which had initial weights of about 180 Gm. The table is divided into two sections, one for the males and the other for the females. Each section is subdivided into three vertical columns. The first two show the weights of the normal controls, the second the mean weights of the guinea pigs given injections of the watery solution of estrogen, and the third row the mean weights of the animals which received the oily solution of estrogen.

Total amounts of 1,040 rat units of estrogen in saline solution and 4,000 rat units of estrogen in oily solution were administered.

TABLE 1—*Variations in Weights of Animals During the Experiments*

	Female Animals			Male Animals		
	Normal	Estrogen in Saline Solution, 1,040 Rat Units	Estrogen in Oil, 4,000 Rat Units	Normal	Estrogen in Saline Solution, 1,040 Rat Units	Estrogen in Oil, 4,000 Rat Units
<i>Light animals</i>						
Initial weight	154	148	153	147	150	149
Weight after 60 days	285	375	365	393	425	420
<i>Heavy animals</i>						
Initial weight	182	182	181	180	180	178
Weight after 60 days	410	50	375	395	407	370

#### MICROSCOPIC OBSERVATIONS

*Epiphyseal Line*—As early as three days after the beginning of the injections, but more definitely after one week's treatment, the zones of endochondral ossification became narrower than ordinarily, and they revealed a slightly increased tendency to calcification.

The first changes observed in the intercartilaginous chondromucoid ground substance were of a retrogressive character. They consisted of a loosening of the matrix, which became vacuolated and later dissolved. The degenerative processes did not spread evenly throughout the epiphyseal line but formed circumscribed, chiefly wedge-like lesions, the base of which was located at the proximal part of the epiphyseal line. These alterations usually occurred after from 240 to 500 rat units of estrogen had been administered. With increasing doses of estrogen and increasing duration of the experiment, the degenerative processes took one of two courses. They became intensified and affected larger areas which ultimately underwent calcification and finally ossification, or they remained more localized, and the areas thus affected became converted

into collagenous hyalinized material which at later stages became ossified without preceding calcification. As a rule, the larger the amounts of estrogen given and the longer the time during which the substance acted, the more advanced was the ossification of the intercartilaginous matrix.

In order to determine more exactly the width of the epiphysial line, a count of the cartilage cells in the epiphysial line of the upper end of the tibia was made, as in our former investigations. The average

TABLE 2—*Ratio of Numbers of Hypertrophic and Columnar Cells in One Row in the Epiphysial Line of the Tibia as Observed in Guinea Pigs Treated with Estrogen for Varying Periods—Smaller Doses*

Period, Days	Total Dose of Estrogen Administered, Rat Units	Light Animals		Heavy Animals	
		Males	Females	Males	Females
3	60	4 10 11	4 9	4 10	4 10
7	120	4 10 9	3 4 7 8*	4 9 10	4 9 10
14	240	4 13 14†	4 13	4 16 17	4 12 13
21	360	2 14 15	0 2 14 15	0 3 14 15	3 4 14
30	520	4 15	3 4 12 13	2 3 15 16	3 14 15
60	1,040	4 16	3 4 8 9	3 4 14 15	2 3 6 7

\* When this guinea pig was killed, it had received 160 rat units

† When this guinea pig was killed, it had received 300 rat units

TABLE 3—*Ratio of Numbers of Hypertrophic and Columnar Cells in One Row in the Epiphysial Line of the Tibia as Observed in Guinea Pigs Treated with Estrogen for Varying Periods—Larger Doses*

Period, Days	Total Dose of Estrogen Administered, Rat Units	Lighter Animals		Heavier Animals	
		Males	Females	Males	Females
3	250	4 10	4 8 9	4 9	3 4 7 8
7	500	4 6	4 8 9	4 8	4 8-9
14	1,000	2 3 14 15	2 3 6 7	2 3 16 17	2 3 13 14
21	1,500	2 3 7 8	2 3 6 7*	2 3 7-8	2 6
30	2,000	3 4 14 15	3 7 8	3 4 9 10	4 15 16
60	4,000	4 8	4 8 9	3 8 9	4 7 8

\* When this animal was killed, it had received 1,250 rat units

number of hypertrophic and of columnar cartilage cells in one row and the ratio between these kinds of cells were established. In normal guinea pigs of corresponding weights, on the average, 4 hypertrophic cartilage cells and 10 columnar cartilage cells were found in one cell row.

Tables 2 and 3 show the individual cell counts and the ratio between the numbers of hypertrophic and columnar cartilage cells after the administration of estrogen. In table 2 are given figures obtained following the injections of the smaller doses of estrogen, and in table 3, those obtained after the injections of the larger doses.

As seen in tables 2 and 3, the narrowing of the epiphysial line in the early stages was due in some instances to a decrease in the number of the columnar cartilage cells. In animals treated with 500 rat units of estrogen in an oily solution the number of the columnar cartilage cells could be as low as 6 to 8, whereas in those treated with 120 rat units of estrogen in a saline solution over the same period there could be a very slight decrease in the number of the columnar cells. The hypertrophic cartilage cells did not show any definite change at this period. After approximately fourteen days a reverse process had set in (fig 1*A*)<sup>21</sup>. The epiphysial line had become distinctly widened. A differential count of the hypertrophic and columnar cartilage cells revealed that this enlargement of the epiphysial line was due to an increase in the number of the columnar cartilage cells, the hypertrophic cartilage cells had become less numerous. The number of columnar cartilage cells in one cartilage row was in some instances as high as 16, the hypertrophic cartilage cells on the other hand, were not infrequently reduced to 2 or to 1, and in some places they were entirely lacking. In most cases the diminution in the latter type of cells occurred in those guinea pigs which had received estrogen in saline solution over long periods. This diminution was found in some animals even after treatment for as long a time as two months, when approximately 1,000 rat units in a watery solution had been injected. Those guinea pigs, however, which had been given the larger doses of estrogen in an oily solution showed this enlargement of the epiphysial line in the great majority of cases for a shorter period of two weeks only. This also coincided with the time when about 1,000 rat units had been administered. In 2 instances, however, an enlargement of the epiphysial line was found after a total dose of 2,000 rat units of estrogen in an oily solution had been injected over a period of one month. The second narrowing (fig 1*B*) of the epiphysial line in the animals which had received more than 1,000 rat units of estrogen in a watery solution took place in the female animals after a period of two months, at a time when, in the corresponding males, the epiphysial line was still wide. A very definite second narrowing of the epiphysial line was found in 6 of 8 animals which had received 1,500 and 2,000 rat units of the oily solution over periods of three or four weeks, and 4,000 rat units of the oily solution, injected over a period of two months, caused a narrowing of the epiphysial line in all instances.

Associated with these changes in the numbers of cartilage cells were certain other changes which affected to some extent also the resting cartilage but concerned mainly the columnar and hypertrophic cartilage

2a Mr S. J. Hayward made the photomicrographs





Fig 1—*A*, photomicrograph of a section of the epiphyseal line of the tibia of a female guinea pig which had received 240 rat units of estrogen in a watery solution over a period of two weeks,  $\times 1448$ . The weight of the animal increased from 180 to 240 Gm. The epiphyseal zone is enlarged. The columnar cartilage cells proliferate. The intercartilaginous matrix is swollen in some areas.

*B*, photomicrograph of a section of the epiphyseal line of the tibia of a female guinea pig which had received 1,040 rat units of estrogen in a watery solution over a period of two months, magnification, same as in *A*. The weight of this animal increased from 185 to 380 Gm. The epiphyseal zone is narrowed. The columnar cartilage cells are diminished in number. The cartilaginous rows are irregular and in some places lacking. The intercartilaginous matrix is increased in amount and sclerosed.

1 *Resting Cartilage* In those cases in which a decrease in the number of proliferating cartilage cells was seen the resting cartilage cells remained inactive. However, with an increase in the number of columnar cartilage cells the zone of the resting cartilage appeared likewise activated. More cells were formed, the darkly stained nuclei assumed an elongated or oval shape, the cytoplasm became irregular, enlarged and thin, and branched processes were sent out by these cells.

2 *Columnar Cartilage* In the cases in which a decrease in the number of cells had occurred the alterations were different from those in cases in which a proliferation of these cells had taken place. In the former the cartilage cells lying in one column had regular outlines, their cytoplasm was narrow, and it contained a dense, rather flattened nucleus. In contradistinction to the normal condition in which the transition from columnar into hypertrophic cartilage takes place by a gradual increase in the size of nucleus and cytoplasm, transitional cell forms were lacking here. However, in individual instances the intensity of these changes varied. In those instances in which the cells multiplied markedly some cells showed progressive hydropic swelling of the cytoplasm (fig 2B) and, owing to the increased pressure within the cells thus produced, the nuclei were pushed toward the periphery, so that not infrequently seal ring cells were formed. Further retrogressive changes led then to complete degeneration and destruction of the cells. In advanced cases whole cell rows were not only injured but in some instances entirely destroyed. This condition, to which were added degenerative processes in the intercartilaginous ground substance caused irregularities throughout the epiphysial line. However, in other instances such degenerations did not occur, instead, the nuclei increased in size and rounded off, and the cytoplasm likewise appeared enlarged. The columnar cells then assumed a spheroid shape, and more and more they tended to take acid stains. At this stage they underwent frequent mitotic division (fig 2B). The maximum of nuclear division was seen when the animals had a weight of from 225 to 275 Gm. Mitoses were found more frequently in the proximal part of the rows of cartilage cells, in the majority of animals they were numerous in the center of the epiphysial line and somewhat rarer toward the peripheral areas. In the beginning, as a rule, the 2 daughter cells occupied the original space of the mother cell and were separated from the upper and lower neighboring cells of the row by a thin but distinctly visible network of intercellular substance. Later on, with further increase in number and size of cells, not only 2 but sometimes as many as 10 and more such acidophilic cells were packed together in one alveolar space, in which no intercartilaginous ground substance was seen.

At still later periods, both the proliferative and the retrogressive changes in the cartilage gradually receded, and then one of two ways



Fig 2—A, photomicrograph of a section of the epiphyseal line of a vertebra of a female guinea pig which had received 1,040 rat units of estrogen in a watery solution over a period of two months, magnification, same as in fig 1 A. The weight of the animal increased from 150 to 375 Gm. The epiphyseal zone is narrowed. Large osseous masses which unite the diaphyseal and epiphyseal bones have replaced the former disintegrated cartilaginous rows. Three osseous plugs are seen in the epiphyseal zone.

B, photomicrograph of a section of the epiphyseal line of the tibia of a female guinea pig which had received 360 rat units of estrogen in a watery solution over a period of three weeks,  $\times 3266$ . The weight of the animal increased from 155 to 250 Gm. Some normal columnar cartilage cells are still visible in the left part of the picture. The majority of the cartilage cells have undergone proliferation, hypertrophy and degeneration. Seal ring cells and cells of epithelioid structures have been formed. Mitosis is noted in the center. The intercartilaginous matrix is increased in amount and shows fibers running in a parallel direction, resembling "asbestos transformation."

of reaction could be distinguished (a) The cell capsules became thickened and at the periphery of the basophilic cells an intensified incrustation with calcium salts was noticeable, which latter subsequently penetrated into the cells. The arrangement of the rows remained irregular. Thus under the influence of estrogen in animals approximately 3 months old and weighing between 350 and 425 Gm the columnar cartilage resembled in histologic appearance in some cases that of normal guinea pigs weighing 700 to 800 Gm and approximately 8 to 9 months old. Or (b) the columnar cartilage cells did not undergo calcification and stained markedly with acid dyes. Then the ossifying matrix began to enclose the cells, and gradually a direct conversion of the cartilage cells into preosteocytes and osteocytes took place. Osseous plugs were formed which extended longitudinally through the epiphysal zone. In the center of these plugs preserved cartilage cells of columnar type could still be recognized. Such cells were also occasionally found in newly formed trabeculae which reached downward into the bone marrow. In these stages the epiphysal line appeared not unlike that of normal animals at the end of the first or in the second year of life.

3 *Hypertrophic Cartilage* The reduction in the number of hypertrophic cartilage cells was due to an accelerated breakdown of the cells and their replacement by bone and it was accompanied by intensified calcification and ossification of the intercartilaginous matrix. If calcification predominated, capillaries of the bone marrow eroded the capsules of the cartilage cells, and numerous epithelioid cells and multinucleated phagocytic giant cells advanced into this region. Subsequently, a quick and accelerated substitution of the calcified cartilage by bone took place. The greater the rapidity of ossification in comparison with the speed of transformation of the columnar into hypertrophic cartilage, the narrower became the layer of hypertrophic cartilage. In cases however, in which the calcification of the hypertrophic cartilage was less marked, the cartilage cells were not broken down, but their direct conversion into osteocytes was observed, a phenomenon analogous to that described in the columnar cartilage. In contrast to the evenly progressing process of replacement, metaplasia of cartilage into bone occurred in an irregular manner in various areas, particularly where thick collagenous acidophilic substances had been deposited in the epiphysal line. It seemed to be the contact of the cells with this collagenous material which gave rise to different centers of ossification. Such centers coalesced in places with the ossified areas in the columnar zone and helped to form the aforementioned osseous plugs (fig 2A).

*Chondrophyte*—Conditions here correspond to the changes which took place in the epiphysal line. In those instances in which hyperplasia

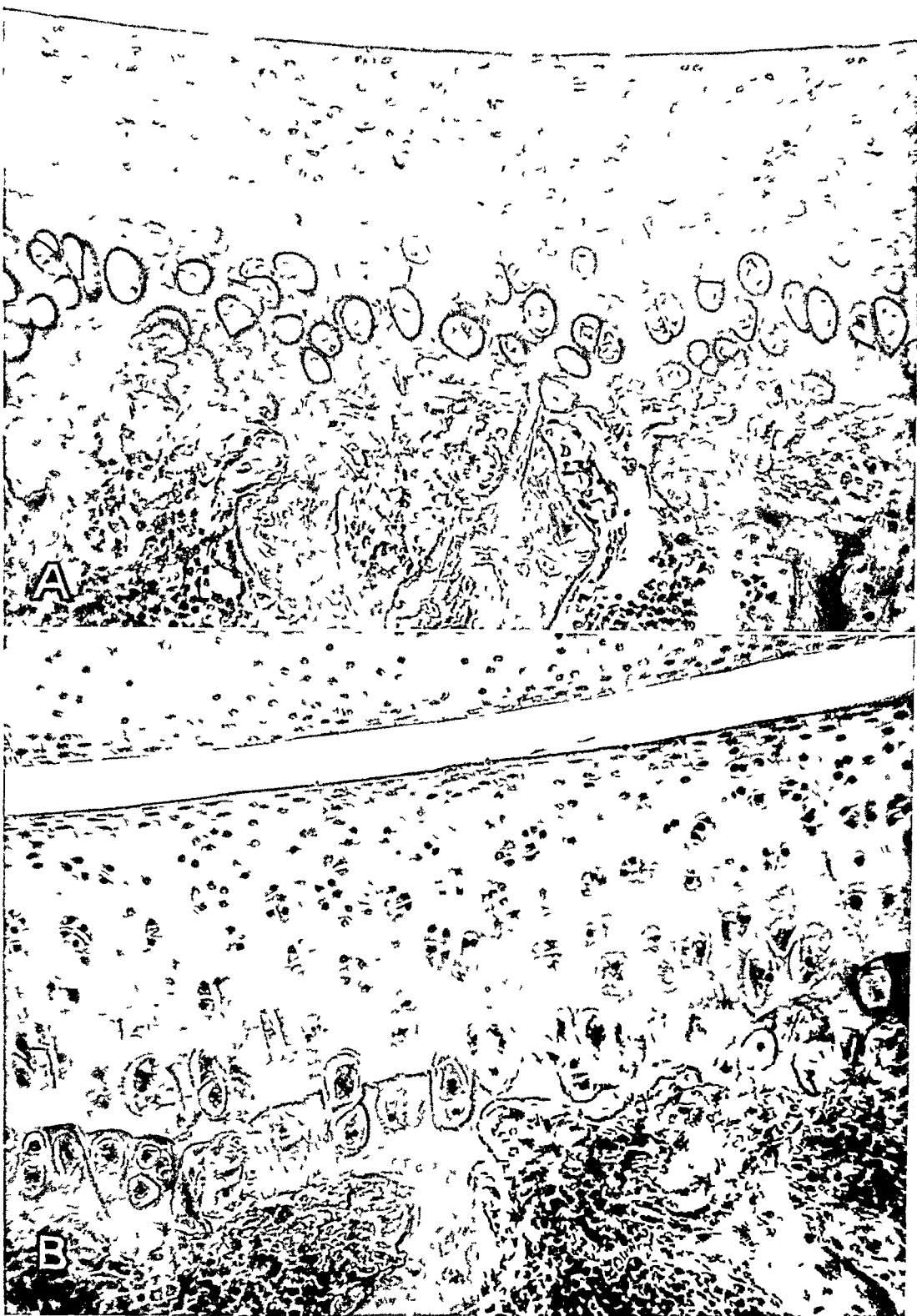


Fig 3—*A*, joint surface of the tibia of a normal female guinea pig,  $\times 220$ . The weight of the animal was 200 Gm. The cells of the various cartilage zones are well preserved. The uppermost or sliding zone shows flat resting cells horizontally arranged. The cells of the transitional zone are somewhat rounded off and likewise resting. The cells of the pressure zone show a fairly regular arrangement. Hypertrophic cartilage cells are present in normal number. Ossification is normal.

*B*, joint surface of the tibia of a female guinea pig which had received 360 rat units of estrogen in a watery solution over a period of three weeks, magnification, same as in *A*. The weight of the animal increased from 155 to 230 Gm. Ossification is advancing and engulfing many hypertrophic cartilage cells. Cells of the pressure and transitional zones hypertrophy and some of them degenerate. Cells of the sliding zone are somewhat hyperplastic. The intercartilaginous stroma is increased in amount and hyalinated.

and hypertrophy particularly of the resting epiphysial cartilage were noted, a slight hyperplasia and hypertrophy of the euhyaline cartilage were also seen in the lateral protuberances, especially in places adjoining the epiphysial line. If the proliferation was very much accentuated, slight retrogressive changes could be detected not only in the cartilage cells but also in the interstitial substance.

*Joint* — After one week's treatment the zone of hypertrophic cartilage was already found to be narrowed, provided as much as about 500 rat units of estrogen had been injected during this period. The intercartilaginous matrix increased more and more, it became sclerosed and at later periods osseous. In contradistinction to the normal processes (fig 3*A*) in which the cartilage cells broke down by way of chondioclasia and subsequently were replaced by bone, in the estrogen-treated guinea pigs not infrequently a direct conversion of the hypertrophic cells into osteocytes occurred. Thus hypertrophic cartilage cells surrounded by bony substance were observed not only in the ossifying layer but also in the trabeculae of the epiphysial cavity. The bony border lamella which demarcates the zone of hypertrophic cartilage was distinctly thickened (fig 3*B*), and gradually the ossification began to advance toward the pressure and transitional zones. At this stage the cartilage cells of these layers became more numerous and enlarged. One cartilage capsule often contained as many as 4 or more large cells, thus indicating that proliferation had taken place. In other instances retrogressive changes were noticeable. The enlarged cytoplasm then became swollen, vacuolated and liquefied, karyorrhexis and karyolysis were frequently seen. The farther the ossification proceeded, the more pronounced became both progressive and retrogressive changes in the cartilage. Within two to four weeks, and this was especially the case in the guinea pigs treated with the larger amount of estrogen in oily solution, thick osseous masses replaced the original hypertrophic and pressure zones. Simultaneously, the normally flat and horizontally arranged cells representing the transitional zone took on a perpendicular arrangement and reacted in the same way as the cells of the original pressure zone. They multiplied and hypertrophied. Slight to moderate proliferation of the cellular constituents of the sliding zone was found in some instances (fig 4). The cartilage of the female guinea pig responded more readily and more intensely to the action of estrogen than that of the male under corresponding conditions. In the males hyperplasia and, in particular, also hypertrophy were less accentuated than in the females.

After the large doses of estrogen had acted for a period of two months, cellular proliferation was no longer seen, however, pronounced thickening of the cortex of the joint was visible. This consisted of

a dense layer of bone lying underneath a narrow sheet of cartilage, which was the remnant of the former covering. Inside the osseous masses the original line of calcification was still present in some places.

*Bone Marrow*—At the earlier stages of the injections of estrogen the marrow contained more fat cells than ordinarily. Chiefly near the endosteum and in the neighborhood of the bony spiculae in the subepiphysial layer, the connective tissue became thickened by the deposition of a larger amount of collagenous fibers, which later under-

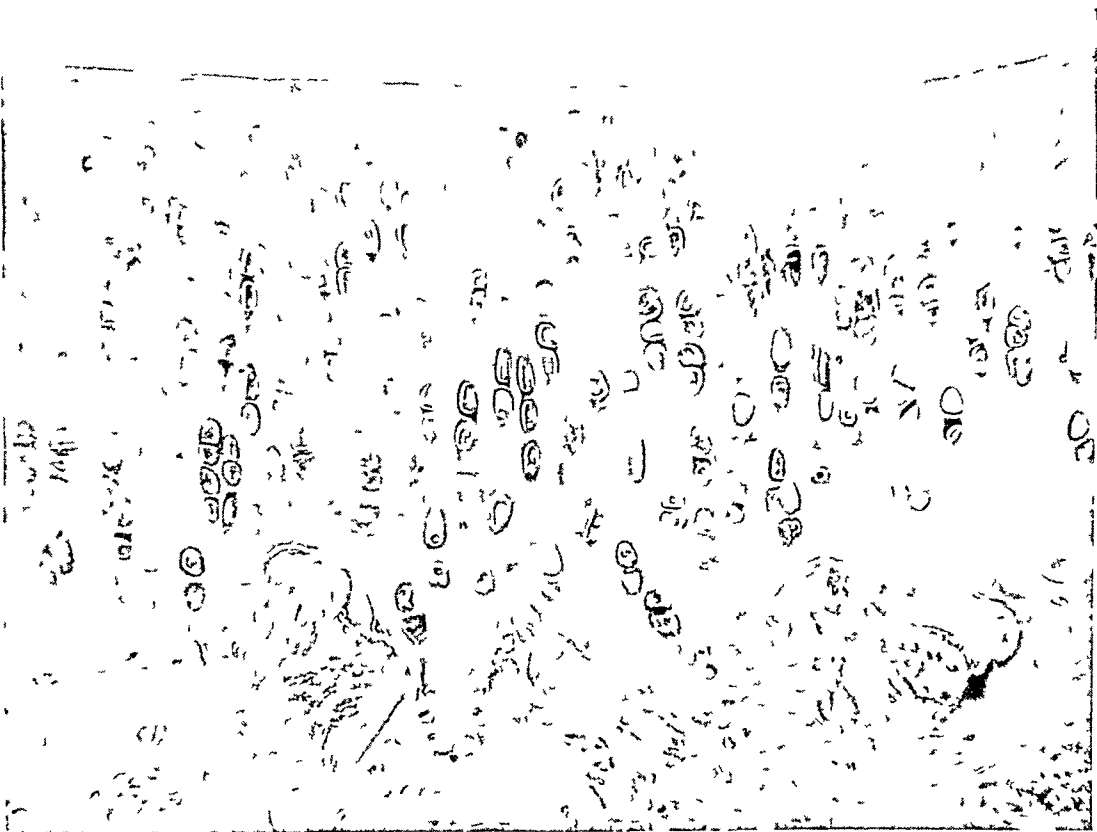


Fig 4—Joint surface of the tibia of a female guinea pig which had received 4,000 rat units of estrogen in an oily solution over a period of two months, magnification, same as in figure 3A. The weight of this guinea pig increased from 150 to 365 Gm. Ossification is far advanced, it has entirely replaced the hypertrophic zone and partly replaced the pressure zone. The cells of the transitional zone are markedly hyperplastic and hypertrophic and show a perpendicular arrangement. Some cells have undergone degeneration. Cells of the sliding zone have partly rounded off. The intercartilaginous matrix contains large masses of sclerosed material, which produce the spotty and uneven appearance of the ground substance in the picture.

went hyalinization and sclerosis. The fibrocytes proliferated markedly, and there were many mitotic figures noticeable. At the same time the mesenchymal reticulum showed accelerated development in the

direction of (a) fibrosis or (b) production of bone-forming elements or (c) production of megakaryocytes

The fibrous changes in the reticulum intensified the fibrosis in the marrow. In some instances the mesenchymal spindle cells rounded off and numerous mitoses appeared in these small to medium-sized ameboid acidophilic epithelioid cells. By accumulation of such cells an osteoid tissue developed. This took place particularly at later stages in the subepiphyseal areas adjoining the layer of hypertrophic cartilage of the epiphyseal zone and of the joint, where in the beginning a fibrous tissue had been laid down. In other places these cells did not assume a tissuelike structure but were arranged in a beadlike manner. This could be observed particularly around the bony trabeculae. In some cases the epithelioid cells acted as osteoblasts which underwent further differentiation into osteocytes, in other instances they coalesced, producing osteoclastic phagocytic giant cells which absorbed and caused the solution of the hyalinized and osseous masses, which latter had been produced in excessive quantities. In the earlier stages of the experiments the appositional processes predominated, as a result of which the number of trabeculae became increased and the individual trabeculae were thickened in the subepiphyseal zone. After one to two months' treatment with estrogen, however, the resorptive processes were comparatively more accentuated. Thus more normal conditions were restored, and in some cases the trabeculae seemed even to be thinner than under ordinary conditions. Farther distally the alterations in these structures were much more moderate, although they were similar in character, near the endosteum, fibrous changes were quite marked. At the end of two months of treatment with estrogen there was no evidence of apposition of bone at the endosteum.

As far as the effect on the megakaryocytes is concerned, the hemocytoblasts were pronouncedly increased in number after three to four weeks of estrogen administration. They frequently underwent fusion and nuclear division. Numerous megakaryocytes were formed, which showed ameboid activity and multiplied mitotically. They were accumulated chiefly in the neighborhood of the trabeculae, where they were held back and at later stages could not be distinguished from the giant cells of epithelioid character.

The question arises as to whether the increase in the number of the megakaryocytes was due solely to proliferation of the cells, or whether it went hand in hand with increased resistance of these young megakaryocytes to injurious conditions and decreased tendency toward disintegration. The latter assumption must be taken into serious consideration, especially in view of the observation made more recently that after administration of estrogen a decrease in number of thrombocytes takes place in the peripheral blood and that severe spontaneous



hemorrhages have been reported in dogs following this decrease in thrombocytes (Arnold and co-workers<sup>3</sup>, Schrade<sup>4</sup>) In one of our animals which had to be put to death we found an extensive spontaneous subphrenic hemorrhage, which may perhaps have been due to thrombopenia

As described, in the epiphysial line some trabeculae might still contain single cartilage cells or groups of cartilage cells, either of the hypertrophic or of the columnar type, which had been directly converted into osteocytes, intensive ossification, however, had taken place in 46 of 48 cases, in only 2 instances the formation of strands of hypertrophic calcified but not as yet ossified, or only incompletely ossified, cartilage was suggested In these instances we had, however, to deal with abnormal conditions One animal had died of peritonitis, the other had had an attack of pneumonia and had suffered loss of weight These 2 cases have necessarily to be discarded for the purpose of analyzing the effects of estrogen on cartilage and bone

*Bony Shaft*—The first changes were seen after one week's treatment and were more marked when higher doses of estrogen had been given The periosteal connective tissue became dense, thickened and sclerosed, it increased gradually in amount and consisted of masses of collagenous and hyalinized fibers, among which were numerous fibrocytes which had proliferated by way of mitoses On the other hand, the osteoclastic giant cells were definitely diminished as compared with the normal conditions, and they were separated from the bone by a layer of fibrous connective tissue Therefore the surface of the compact bone was smoother than is ordinarily the case, and only a few, quite superficial lacunar grooves were visible This was the state of the periosteum at the period when appositional processes predominated along the trabeculae in the bone marrow At later periods there appeared within the periosteal connective tissue, medium-sized to large round epithelioid acidophilic cells, which in some areas were arranged in small islets and longitudinal groups and in other places had coalesced and formed giant cells One has the impression that the accumulations of such cells were the consequence of the proliferation and hyalinization of the connective tissue The latter processes inhibited the ameboid movement of the epithelioid cells, which were held back and could not come in contact with the compact bone

After administration of estrogen for periods of one month and over, large round and spindle-shaped cells, representing various transitional structures between fibrocytes, precartilaginous cells and

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3 Arnold, O, Hamperl, H, Holtz F, Junkmann, K, and Marx, H  
Arch f exper Path u Pharmacol **186** 1, 1937

4 Schrade, W Folia haemat **61** 145, 1939

typical euhyaline cartilage cells, were found in the periosteal connective tissue. The closer these cells were situated to the compact bone, the more they enlarged and took on the definite shape of cartilage cells, in which the cytoplasm often was very much vacuolated (fig 5A). In some places, chiefly in the middle of the shaft, such cartilage cells were found within the osseous masses, they seemed to have invaded and dissolved the compact bone. Between these cells there remained large strands of dense acidophilic substances. The cases in which these marked processes of invasion and solution of the bone took place were those in which also around the trabeculae of the marrow the resorptive changes were more pronounced than the appositional ones. The development of cartilage cells along the shaft was found in all the 8 animals which were examined after two months of treatment with estrogen and in the majority of those examined as early as after one month. Although in some instances the haversian channels seemed to be less frequent than ordinarily, no evidence of an appreciable apposition of bone could be detected after two months' treatment.

*Ribs*—In the cartilaginous part of the ribs the resting cartilage degenerated in direct proportion to the amount of estrogen administered and the time during which it acted. The cytoplasm of the cells appeared enlarged and vacuolated. The nuclei became pyknotic and finally disintegrated. Thus changes which under normal circumstances are seen in old guinea pigs became more and more intensified. In the beginning, only single cells were affected, but later whole groups of cells underwent this retrogression. The intercartilaginous matrix became first swollen and vacuolated, at later stages it increased in amount, and especially around the cytoplasm of the degenerating cells acidophilic collagenous and hyalinized substances were seen covering circumscribed areas and including more or less preserved cartilage cells or cell debris (fig 5B). In cases in which the process was advanced such sclerosed material resembled osseous ground substance, although the enclosed cells could still have the characteristics of cartilaginous elements. This condition differed from that seen in normal old guinea pigs, inasmuch as under the influence of estrogen hyalinization of the degenerated cartilage occurred, whereas calcification of the degenerated areas was lacking. In the zone of proliferating cartilage the matrix and the cells occasionally underwent similar, although less marked retrogressive alteration, but at later periods, especially, growth processes became noticeable. The columnar cells multiplied frequently by way of amitotic division. The layer of the hypertrophic cartilage was somewhat narrowed, and the cells showed only a moderate degree of calcification. On the other hand, ossification proceeded with the same rapidity and intensity as in the epiphyseal line of the long bones. At the periosteum, chiefly near the chondio-osseous junction thickened



Fig 5—*A*, section from the shaft of the tibia of a male guinea pig which had received 1,040 rat units of estrogen in a watery solution over a period of two months,  $\times 2124$ . The weight of the animal increased from 180 to 435 Gm. At the top there is the bony shaft with marrow spaces. At the bottom there is the proliferating connective tissue of the periosteum. Between periosteum and bone there is an enclosure of cartilage which, below the marrow cavity at the right end of the picture, is seen penetrating into the bone. Some of the cartilage cells have undergone hypertrophy. Cystic enlargement of the cartilage in the middle of the picture is noted.

*B*, section through the cartilaginous part of a rib of a female guinea pig which had received 520 rat units of estrogen in a watery solution over a period of one month, magnification, same as in *A*. The weight of this animal increased from 175 to 265 Gm. Marked hyalinization of the intercartilaginous matrix of the rib is indicated by the dark-appearing material.

hyalinized fibrils developed in the intercellular stroma. The fibrocytes proliferated markedly and were quickly converted into precartilaginous cells. Finally, owing to the stimulation of growth processes in the periosteal tissue, a solution of the bony substance could take place. As far as the periosteal changes as well as those affecting the other constituents of the ribs were concerned, one had to deal with structural alterations which were identical with those in the corresponding elements of the tibia. As a consequence of these various processes, enlargement and thickening, especially of the cartilaginous parts, resulted, which in advanced conditions produced a slight rosary-like swelling of the chondio-osseous junction. The behavior of the bone marrow corresponded to the findings in the long bones.

*Vertebral Column*—In the intervertebral disks the mesenchymal stroma underwent retrogressive changes, which were indicated by loosening and vacuolation of the ground substance. Subsequently marked thickening and hyalinization of the fibrils were noticeable. At later stages the sclerosed matrix, which contained bundles of thick collagenous fibers, became distinctly denser than ordinarily and increased in amount. Alterations of the cells went hand in hand with changes in the stroma. The spheroid fibrocartilage cells, which usually are scarce, began to proliferate by way of amitotic division. They increased not only in number but also in size. The cell nuclei were enlarged, and the cytoplasm was rounded off. Thus in some cases structures resulted which showed all kinds of transitions between ordinary connective tissue cells and cartilage cells and which finally could not be distinguished from cells characteristic of euhyaline cartilage, in other instances the hyperplasia led to the production of dense connective tissue. Within three to four weeks after the beginning of the administration of estrogen pyknosis and karyolysis of the fibrocartilage cells could be observed frequently. Retrogressive changes were accentuated the more, the more the cells increased in size.

The effects noted on the vertebrae corresponded in all details to those seen in the long bones. As to the zones of endochondral ossification, degenerative processes were followed by hyalinization of the intercartilaginous chondromucoid matrix and its conversion into thick collagenous acidophilic masses, which became similar to osseous ground substance. Owing to predominance of ossification processes over new formation and differentiation of cartilage cells, the epiphysial line became narrowed at first. Subsequent growth, in particular of the columnar cartilage cells, as evidenced by nuclear divisions associated with cellular hypertrophy, led to temporary enlargement of the zones of ossification. Mitotic proliferation of the euhyaline cartilage cells covering the intervertebral joints was observed in cases in which nuclear division had occurred also in the epiphysial cartilage cells. Ultimately,

whole cell rows of proliferating cartilage degenerated and broke down. Such degenerated areas were quickly replaced by bone. Thus numerous thick wedge-like osseous plugs were found extending from the marrow throughout the whole epiphysis (fig 2A). These plugs occasionally contained cartilage cells which were still recognizable and which were being converted into osteocytes.

After one to two months' treatment with estrogen these plugs became so large and numerous that the epiphysial line appeared almost ossified. The processes of apposition and solution which took place at the inner border of the periosteum and around the trabeculae of the bone marrow were comparable to those seen in the long bones, likewise, the changes in the bone marrow and its various elements were the same.

#### COMMENT

The action of estrogen on cartilage and bone in the immature guinea pig is shown as follows. At first, loosening and vacuolation of the intercartilaginous chondromucoid ground substance take place. This condition is associated with temporary suppression of the differentiation and growth of the resting and proliferating cartilage, whereas the ossification of the hypertrophic cartilage is accelerated. As a result of these changes, the zones of endochondral ossification become narrower. In the joints the layer of hypertrophic cartilage also diminishes in width and subsequently undergoes ossification. The bone marrow becomes fatty, an apposition of epithelioid osteoblasts along the trabeculae causes thickening of the bony spicules which may also increase in number. In the subepiphysial layer, at the endosteum and in the periosteum intensified growth processes of the connective tissue lead to development of a fibrous tissue in which numerous collagenous fibers are sclerosed. The retrogressive changes which gradually progress in the cartilaginous matrix may also affect the cartilage cells themselves, karyolysis and karyorrhexis occur, and a loss of the normal structure of the epiphysial line results. Later, the growth processes of the euhyaline cartilage are resumed, hyperplasia and hypertrophy are increased above the normal level. Thus the zones of endochondral ossification and the cartilaginous covering of the joints appear widened. In the cartilage cells of the joint the hyperplastic and hypertrophic changes are distinctly more accentuated in the female than in the male guinea pigs. In the various cartilaginous tissues the sclerosed intercellular matrix increases in amount and becomes more and more similar to osseous ground substance. With increase in the production of sclerosed connective tissue, the ameboid movement of the osteoblastic epithelioid cells in the neighborhood of the trabeculae of the bone marrow and in the periosteal cambium layer is inhibited, and in the osseous structures solution processes predominate over the apposition of bone elements.

At still later stages, in the zones of endochondral ossification either the proliferating cartilage undergoes calcification, breakdown and replacement by bone or irregular circumscribed accelerated metaplastic conversion of columnar and hypertrophic cartilage cells into osteocytes leads to the formation of osseous plugs. This condition causes a second narrowing with partial ossification of the epiphysal lines and complete ossification of the cartilage cells of the pressure and transitional layers in the joints. On the other hand, the bony constituents of the marrow cavity assume again more normal appearances. The formation of cells resembling hemocytoblasts as well as of megakaryocytes is increased. In the periosteum, conversion of connective tissue cells into hypertrophic precartilage and ultimately into typical euhyaline cartilage cells is noticeable. These cartilage cells which are deposited along the bony compact layer of the shaft invade the osseous substance, they may undergo marked hypertrophy and also some degeneration.

The degree of the changes thus induced varies in different cases and is determined by the time during which the estrogen acts, the amount of the substance administered and the speed of resorption of this substance.

We must then conclude that the effect of estrogen on the skeletal tissues is a rather complex process. It cannot be defined as mere ossification of the epiphysal disks with subsequent cessation of growth in the lengthwise direction, as described by Tausk and de Fremery<sup>5</sup> in castrated dogs treated with an estrogen for twelve days, nor as hyperostotic osteoclerotic growth in the shaft and the marrow cavity of the long bones, changes which have been observed by Zondek<sup>6</sup> in fowls and by Gardner and Pfeiffer in mice<sup>7a</sup> and pigeons<sup>7b</sup>.

As far as the effect on the epiphysal line is concerned, a fairly general agreement seems to exist that estrogen produces narrowing and subsequent closure of this structure. Zondek found persistence of the epiphysal disks with simultaneous arrest of growth of the long bones, a condition which he identifies as pituitary dwarfism caused by inhibiting action of the estrogen on the pituitary gland. Only two investigations have come to our attention in which a histologic analysis of the epiphysal changes was made. One of them was carried out by Coryn,<sup>8</sup> who administered gonadotropic substances to castrated rabbits, and the other by Seeman<sup>9</sup> who, on the assumption that the narrowing of

5 Tausk, M., and de Fremery, P. *Acta brev. Neerl.* **5** 19, 1935.

6 Zondek, B. *Lancet* **2** 842, 1936.

7 Gardner, W. U., and Pfeiffer, C. A. (a) *Proc. Soc. Exper. Biol. & Med.* **37** 678, 1938, (b) *Endocrinology* **23** 485, 1938.

8 Coryn, G. *Presse med.* **45** 1649, 1937.

9 Seeman, H. *Endokrinologie* **18** 225, 1937.

the epiphysial line under the influence of estrogen was definitely proved, used this effect as the basis of a test for the presence of estrogen in the testicles of hogs. However, it may be stated that not only estrogen but also an extract of the anterior lobe of the pituitary gland may lead to narrowing of the epiphysial line<sup>10</sup> and that, as our experiments have shown, estrogen at certain stages may, on the contrary, cause widening of the zones of ossification.

As to new formation of bone, we have observed accelerated and intensified ossification in the preexisting zones of ossification and also in the cartilage of the joint. The same observation seems to apply also to the cartilage of the ribs, where the hyalinizing processes may perhaps be considered as precursors of ossification. But this interpretation will have to be verified by further experiments, which must be extended over longer periods.

However, with the exception of temporary apposition of bone around the trabeculae and likewise temporary increase in the number of the trabeculae in the epiphyses and diaphyses during the first four weeks of treatment, we have not observed any appreciable apposition of bone, either periosteally or endosteally, sufficient to cause thickening of the compact bone or narrowing and occlusion of the lumen of the marrow cavity. On the contrary, we have seen resorptive processes of considerable intensity acting on the cortex of the bones as well as on the trabeculae of the bone marrow after the first month of treatment.

In attempting to interpret the action of estrogen on the skeletal tissues we have (1) to distinguish between the effects of this substance on the various cellular constituents and those on the intercellular matrix and (2) to determine, if possible, which of the changes described are produced by the hormone itself, which may be due to a secondary hormonal influence and which may be induced by altered conditions in the affected tissues. It must be borne in mind that ossification even under normal circumstances is constantly controlled by both general and local factors and that a disturbance in either may alter the course of ossification.

Two different effects are exerted by estrogen on the cartilage cells of the epiphysial lines. At early stages and again after considerable amounts of the estrogen have been administered, definite suppression of growth occurs as far as size and number of cells are concerned. It is possible to explain this as a reversal of the effect of gonadectomy, which latter stimulates the growth of the cartilage by way of the pituitary gland.<sup>1</sup> Estrogen may inhibit the effect of the anterior lobe of the pituitary gland in a nonspecific way.

This explanation, however, cannot hold good for the second effect, observed in the intermediary stage, in which the epiphysial line is

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<sup>10</sup> Silberberg, M. *Proc Soc Exper Biol & Med* **32** 1423, 1935

widened and the number of the columnar cells is increased. At present we cannot harmonize these opposite effects with each other unless we attribute the last named to local conditions. The degenerative changes which take place within the matrix may perhaps exert a stimulating influence on neighboring cells, causing them to multiply and to assume the appearance of epithelioid cells. This condition persists up to the point when ossification advances from the marrow and prevents the cartilage cells from further proliferation, it is found also in the cartilage of the joints, where the advancing hyalinization and ossification of the matrix is accompanied by growth processes of the cartilage of the adjoining zones.

In the intercartilaginous matrix initial swelling and vacuolation are followed by marked hyalinization and subsequent ossification. In this connection it may be of interest to mention that hyalinization of the connective tissue and muscle tissue of the uterus and vagina of the mouse has been observed by Loeb, Suntzeff and Burns<sup>11</sup> following injections of large quantities of estrogen and that this process initiated foreign body reactions in the surrounding connective tissue. The question arises as to whether the hyalinization of the cartilaginous matrix, which initiates the metaplasia of the cartilage cells into osteocytes, is to be considered as the first stage of the process of ossification or whether it creates merely a local condition which in the case of the tissues possessing the ability to form bone is favorable to increased production of bone.

The rather widespread resorptive processes, which occur at the cortex with the cooperation of periosteal cells and about the trabeculae under the influence of connective tissue constituents of the bone marrow, are an expression of destructive action counterbalancing the bone building functions which these cellular elements exert under other conditions. We have observed a great number of giant cells, which dissolve the bony substance. The connective tissue may penetrate rather deeply into the cortex and here form small plugs surrounded by bone. In these areas as well as in others adjoining the bone from the periosteal side, cartilage is frequently found. We cannot as yet decide whether this intense ectopic cartilage formation is induced by hormonal factors or whether it is a consequence of local conditions, the nature of which needs further investigation. In this connection we may refer to the observations of Zawisch-Ossenitz,<sup>12</sup> who described the occurrence of islands of "basophilic substance" in the diaphysis of the long bones in various species of animals. She refers to the similarity which this basophilic substance exhibits to precartilaginous substance.

11 Loeb, L., Suntzeff, V., and Burns, E. L. *Science* **88** 432, 1938, *Am J Cancer* **35** 159, 1939.

12 Zawisch-Ossenitz, C. *Ztschr f mikr-anat Forsch* **10** 473, 1927.



## SUMMARY

Estrogen administered to immature guinea pigs over periods of from three to sixty days causes retrogressive changes, increased hyalinization and ossification of the intercartilaginous ground substance in the epiphysial disks, ribs and vertebrae

The cartilage cells respond with temporary cessation of growth and differentiation, followed by degenerative changes and subsequent proliferation and hypertrophy. The growth processes are accompanied by an accelerated and intensified breakdown of the growing cartilage and replacement by bone or by an accentuated direct metaplasia of columnar and hypertrophic cartilage cells into osteocytes. These processes may be considered as premature aging of the cartilage.

A transitory deposition of osteoid tissue around the preexisting trabeculae is followed by resorptive processes of the bony substance. A strong proliferation of the mesenchymal stroma of the bone marrow and the periosteum leads to the development of a sclerosed fibrous tissue. At present it is not certain that these effects are specific to certain hormones. One may also consider the possibility that in addition local factors play a role in the development of these processes.

# THE ANITSCHKOW "MYOCYTE"

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In 1913 Anitschkow<sup>1</sup> published a report of his experimental studies on the formation of granulation tissue in the myocardium. In this paper he discussed at some length a cell in the myocardium to which he gave the designation "myocyte." This cell was one of characteristic and constant appearance. It was distinguished by the presence of a peculiar longitudinal bar or rod within the nucleus, composed presumably of condensed chromatin substance, this bar had a serrate edge and was separated from the nuclear membrane by a clear zone on all sides (fig 1A).

Anitschkow's attention was drawn to this cell by its occurrence in granulation tissue which had been experimentally produced by introducing foreign bodies into the myocardium of rabbits. This "myocyte" had previously been observed and superficially described, particularly by Von Oppel<sup>2</sup> in 1901 and Saltykow<sup>3</sup> in 1905. Both these investigators and also a number of subsequent observers, including chiefly Karsner and Dwyer,<sup>4</sup> Jacki,<sup>5</sup> Watjen,<sup>6</sup> Hesse and Hesse<sup>7</sup> and Semsroth and Pool,<sup>8</sup> had their attention drawn to the "myocyte" in the course of studies which were concerned with the reaction of the myocardium to traumatic injuries, foreign bodies, inflammation and infarction produced by ligation of coronary arteries and also with the question of regeneration of the myocardium following its destruction from one cause or another.

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From the Laboratories of Lebanon Hospital and Mount Sinai Hospital

1 Anitschkow, N. N. *Beitr. z. path. Anat. u. z. allg. Path.* **55** 373, 1913

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5 Jacki, E. *Frankfurt Ztschr. f. Path.* **22** 82, 1919

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7 Hesse, M., and Hesse, E. *Virchows Arch. f. path. Anat.* **252** 275, 1924

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In 1929 there appeared a paper by Wenezianowa-Grusdkowa<sup>9</sup> devoted specifically to the Anitschkow "myocyte," in which the question of the origin of this cell was considered from the standpoint of modern histogenic concepts and in which a more thorough attempt was made to discover the nature of the cell by histologic studies and by intravital injections of dye

In 1938 Richard H Jaffe,<sup>10</sup> in his monograph on the reticuloendothelial system, presented a rather complete though short, discussion of the Anitschkow "myocyte"

In the present study the question of the origin and significance of the Anitschkow "myocyte" is taken up anew on the basis of embryologic and comparative anatomic observations

#### MATERIAL AND METHODS

For the purpose of this study, numerous hearts were examined in the following general groupings (1) human age series, (2) human embryo series, (3) chick embryo series and (4) general animal series. The animal series comprised hearts from over twenty-five different species and included almost the entire subphylum of vertebrates. The hearts were examined chiefly in thin paraffin sections stained with hematoxylin and eosin and occasionally with other stains, such as Van Gieson's and the silver impregnation stain. In addition, representative sections were made of fresh unfixed material prepared by the frozen section method and stained with hematoxylin and eosin. Also, frozen sections of fixed material were examined. Fixation was always by formaldehyde.

Because of the distinctive intranuclear formation present in the cells under consideration and because of the occurrence of these cells in a rhythmically pulsating organ, namely, the heart, the possibility of artefactual influences determining their appearance in sections was taken into consideration. However, examination of fresh unfixed material by the frozen section method and examination of thin blocks of fresh unfixed tissue prepared without freezing and examined by the Terry method (utilizing polychrome methylene blue) disclosed intranuclear formations identical with those observed in fixed paraffin sections. Semsroth and Pool<sup>8</sup> also felt that the distinctive nuclear appearance was not an artefact, chiefly because other nuclei in the vicinity were of normal appearance.

#### GENERAL DESCRIPTION

This investigation has been concerned with a cell (fig 1A) which shows a characteristic appearance under a high power objective. This appearance is very striking, and in the vast majority of instances it is clearly distinguishable from that of all other nuclei occurring not only within the heart but, so far as our experience goes, in other tissues of the body as well. In the normal heart during the postnatal period, in both man and animals, this cell appears practically free from distinguish-

9 Wenezianowa-Grusdkowa, M S. Frankfurt Ztschr f Path **37** 538, 1929

10 Jaffe, R H. The Reticulo-Endothelial System, in Downey, H. Handbook of Hematology, New York, Paul B Hoeber, Inc, 1938, vol 2, pp 1008 and 1089

able cytoplasm. Occasionally a small perinuclear zone of cytoplasm may be observed at either pole of the cell (fig 1 C). Such cytoplasmic tabs, when present, are clear, homogeneous and slightly basophilic and taper out rapidly into thin strands at each end of the cell, which disappear out rapidly into thin strands at each end of the cell, which disappear

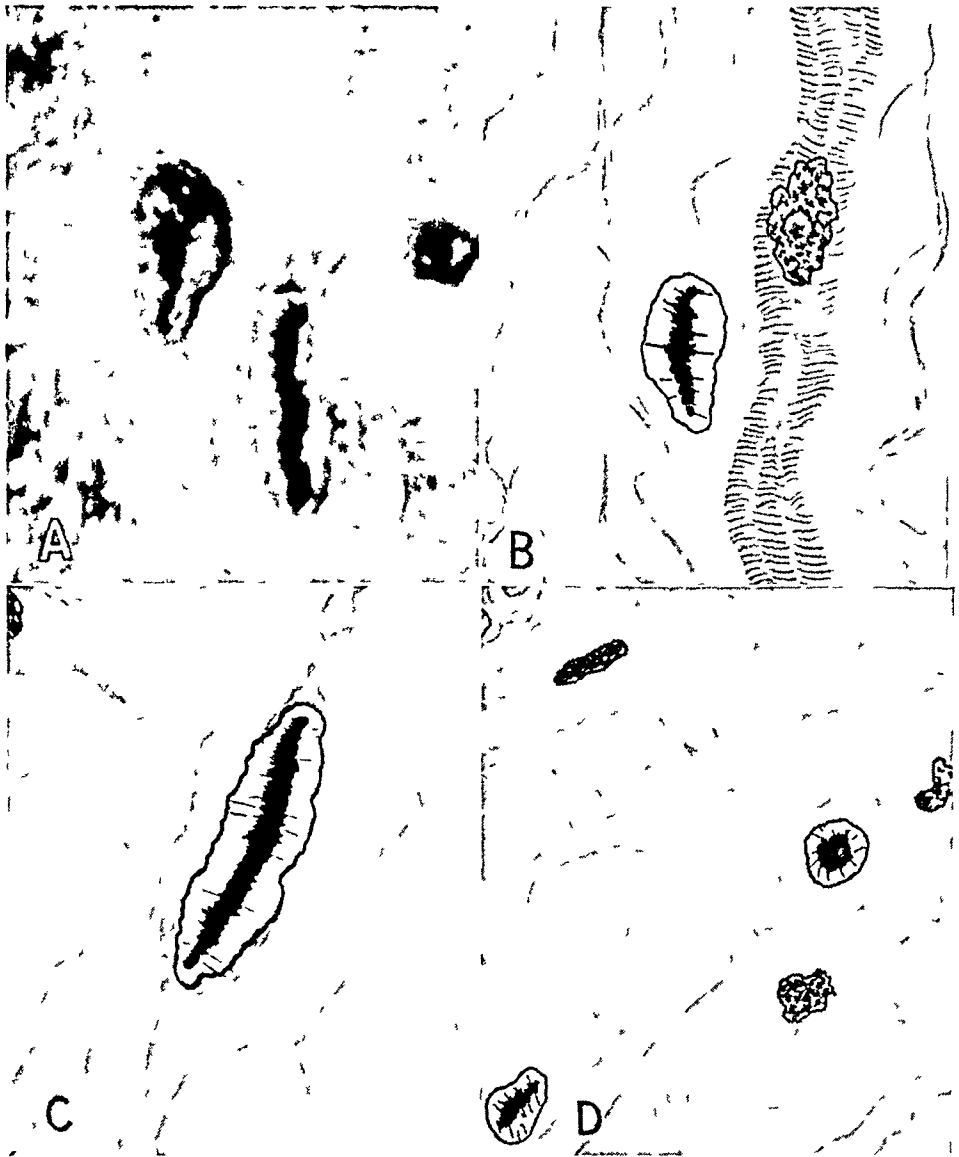


Fig 1—*A*, human heart valve at the age of 8 years, photomicrograph,  $\times 1,800$ . Two "myocytes" are seen sectioned longitudinally. *B*, human heart at the age of 6 months, camera lucida drawing,  $\times 1,800$ . A "myocyte" in longitudinal section adjoins a muscle fiber. *C*, adult frog heart, camera lucida drawing,  $\times 3,000$ . A "myocyte" is seen in longitudinal section, with tabs of cytoplasm visible at both poles. A portion of a fibroblast is seen above to the left. *D*, human heart at the age of 6 months, camera lucida drawing  $\times 1,200$ . At the right of center is a "myocyte" in cross section. A "myocyte" in longitudinal section is seen at the lower left. Note the three muscle nuclei within muscle fibers.

appear into the ground substance. In the experiments of Wenezianowa-Grusdkowa,<sup>9</sup> the cytoplasm of the "myocytes" was clearly indicated in those instances in which these cells stored intravital dye.

The special morphologic characteristics of this cell are concerned with the nucleus. The appearance presented is almost constant in any given plane regardless of the part of the heart in which the cell is encountered or of the prenatal or postnatal age period in which the heart is examined. The nucleus as seen in longitudinal section appears as a rather uniformly elliptic structure with a sharply outlined, thin uniform membrane, which stains darkly with hematoxylin. The intranuclear space appears empty or vacuolated, and within this clear zone, occupying the central portion of the intranuclear space, there is a conspicuous rod-shaped body. This body varies somewhat in thickness, being usually tapered toward the poles, and its outline is slightly serrated. This body always takes a deep nuclear stain. The general configuration of this rod is such that it occupies almost the entire long axis of the nucleus, reaching at the tapered poles almost to the nuclear membrane. At its widest point, which is usually near the center, the width of this rod-shaped structure is approximately one-third that of the nucleus, and because of its central location there remains on each side of it a clear zone which is equal also to approximately one-third the width of the nucleus. Radiating from the serrated edges of the rodlike body, usually at right angles to its long axis, are extremely fine fibrillar structures which extend directly from the side of the rod-shaped body toward and in many cases up to the nuclear membrane. These threadlike structures cannot be observed emanating from all the toothlike projections in this intranuclear structure but are quite evenly distributed at short intervals. Within the nucleus no other constant chromatin elements or nucleoli can be observed, and those parts of the intranuclear space which are not occupied by this intranuclear body or by its radiating strands appear entirely clear.

In cross section the nucleus appears as a small circle in the center of which is a dark circular body with a serrated edge from which strands radiate toward and to the nuclear membrane. The appearance of these strands indicates that they radiate from the surface of the intranuclear body in all planes (fig 1 *D*). We have occasionally observed a lighter staining zone in the center of the intranuclear body.

Occasional specimens, chiefly the 18 day embryo chick heart, presented clusters of these cells with abundant cytoplasm (fig 2 *A*). Such cells were stellate or polygonal in shape, with a rather abundant amount of clear homogeneous basophilic cytoplasm. The stellate projections of cytoplasm tapered off into fine fibrillar strands and were lost in the surrounding ground substance.

Similar cytoplasmic inclusions have been observed by us in human hearts—for example, in rheumatic fever. We have on rare occasions observed nuclei of suggestively similar structure in other tissues. In an instance of lipoid pneumonia in a child, with metaplastic changes in the tracheobronchial mucosa, the metaplastic tissue presented one epithelial cell of similar structure. Occasionally the nuclei of cardiac muscle fibers when there are pronounced longitudinal folds in the nuclear membrane may bear a superficial resemblance to the cell described. This artificial resemblance is quickly clarified by study of a cross section of the nuclear membrane. We have not made any extensive examinations of other tissues of the body from the standpoint of whether or not similar cells may be present outside of the heart, although in our general experience with human tissues, such cells have not been encountered. Nevertheless it is possible that further studies might reveal similar or identical nuclei outside the heart.

#### GENERAL DISTRIBUTION

In an average normal human heart, for example, during the third decade of life "myocytes" may be found distributed throughout the entire musculature. Within the ventricles, in the intraventricular septum and in the papillary muscles, extending all the way up to the auriculoventricular ring, these cells are frequently encountered with the high power objective. Also in the auricular musculature and in the substance of the mitral, tricuspid, aortic and pulmonary valves these cells are found. All four valve rings contain numerous "myocytes."

Within the myocardium, they may be found most easily by searching in the interfascicular planes. These planes, in which the larger blood vessels run before their break-up into the capillaries which ramify within the cardiac muscle fasciculi, are composed of loosely arranged collagen bundles and argyrophilic fibers. By searching along such planes, occasional "myocytes" will be encountered, in some areas more than in others, along with the usual cellular elements present in this tissue, namely, fibroblasts, occasional macrophages, a rare mast cell and the nuclei of arterioles, venules and lymphatics, with their perivascular cells. The nucleus of the "myocyte" in such an area is so arranged that its long axis is usually parallel to the collagen fibers running in these planes. The "myocytes," however, are not confined to the interfascicular planes but may be found also within the muscle fasciculi. In such instances they lie in close proximity to individual cardiac muscle fibers (fig. 1 *A* and *B*). In many instances, they appear to lie within these fibers, although examination of the cross section reveals this to be the result of superposition or infolding. For the most part, "myocytes" lie external to the sarcolemma of the cardiac muscle fibers.

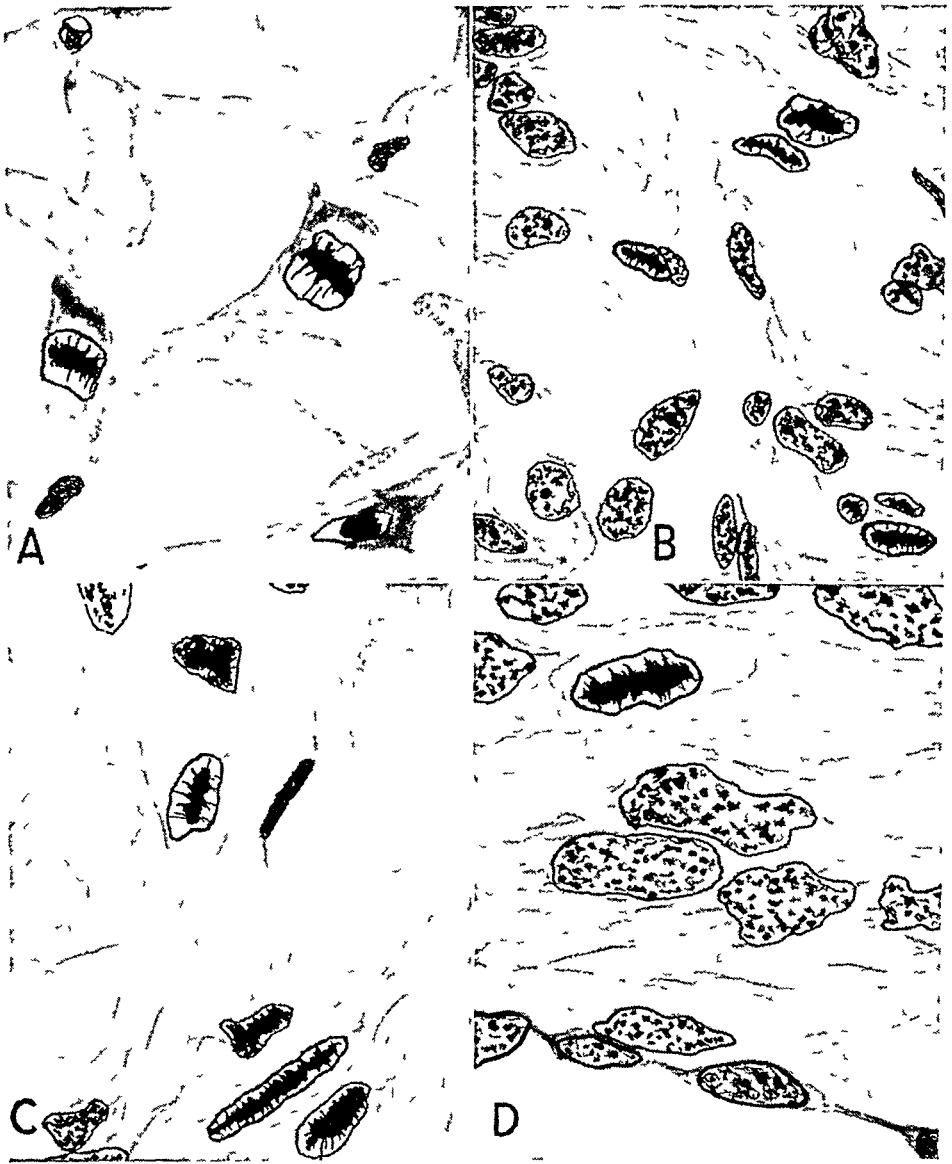


Fig 2—*A*, chick embryo heart at 18 days, camera lucida drawing,  $\times 1,200$ . An area in the valve cushion is shown in which “myocytes” may be seen with distinct cytoplasm containing zones of basophilia and tapering off into the surrounding ground substance. *B*, rabbit embryo heart at about 20 days, camera lucida drawing,  $\times 950$ . An endocardial indipping in the ventricular wall is seen. To the left of center is a “myocyte” closely applied to an endothelial cell nucleus. Striations are not yet visible in the cardiac muscle. Numerous small and large muscle nuclei are present and several “myocytes.” *C*, adult fish (carp) heart, camera lucida drawing,  $\times 950$ . Distinct “myocytes” are present. A muscle fiber is seen above to the right. *D*, human embryo heart at 1 month, camera lucida drawing,  $\times 1,400$ . A “myocyte” is seen a short distance from the endothelium lining the primitive ventricle. Large muscle nuclei and immature muscle fibers are present. Muscle striations are not yet visible.

In the cardiac valve these cells are occasionally encountered in the fibrous body of the valve along with other common cells such as fibroblasts and occasional macrophages and round cells

Many "myocytes" may be found beneath the endocardium not only in the heart valves but also elsewhere in the heart, where they may be traced following the complex indippings of the endocardium among the muscular trabeculae of the ventricles (fig 2B)

In the human embryo series and in a series of 20 chick embryos, aged 1 to 21 days, "myocytes" were found with ease. In the chick embryo heart, they were observed first on the twelfth day, and the youngest human embryo heart in which these cells were observed was about 1 month old. In both these embryos the earliest "myocytes" were found immediately beneath the endothelium of the ventricle and appeared to be related to the distribution of endocardium, inasmuch as they were found first not only beneath the endothelium in the ventricles but also in those endocardial aggregations which constitute the valve cushions. For example, in early embryos, with valve cushions in the earliest stage of their formation, the main substance of these cushions contained relatively large numbers of "myocytes." Indeed, the substance of the valve cushions and later the valves themselves contained large numbers of "myocytes" throughout the entire stage of embryonal development (fig 2A). Within the myocardium the appearance of these cells and their penetration throughout the muscle seem to follow the complicated endocardial indippings which permeate the entire myocardium during the earliest stages of the development of the heart, and it appears probable to us that this cell reaches all parts of the myocardium during this stage of development (fig 2B and D). By the fifth month, in the human embryo, large numbers of "myocytes" may be found throughout the myocardium, although their numbers appear to be relatively diminished during the later stages of embryonal life.

As regards the general animal series, "myocytes" were found without difficulty in all hearts examined<sup>11</sup> (See fig 1C for the frog, fig 2B for the rabbit and fig 2C for the fish.)

Examination of human hearts of the first to the eighth decade of life showed a rather constant quantitative and qualitative distribution of "myocytes."

#### COMMENT

The foregoing anatomic observations indicate that the "myocyte" is a cell of distinctive morphology, whose development and topographic relationships suggest that it constitutes part of the supporting framework of the myocardium and valves

<sup>11</sup> Hearts were studied in the following animals: fish (carp), amphibians (frog), reptiles (boa, turtle, iguana, lizard), birds (chicken, crane, ibis, pelican, owl, parrot, hawk), and mammals (armadillo, polar bear, tahr, sheep, sea lion, rabbit, guinea pig, cat, monkey [mandrill, capuchin, marmoset, macaque]).



This conclusion is based on the location of these cells in the interfascicular planes, in the fibrous core of the cardiac valves and within the muscle fasciculae between the individual cardiac muscle fibers, i. e., external to the sarcolemma fibers. Furthermore, the polygonal or stellate form, tapering out into fine cytoplasmic fibrillae (when cytoplasm is observed), conforms to the general morphology of interstitial cellular elements. They do not appear, from our studies, to bear any relationship to specially differentiated myocardial fibers (Purkinje system). Because they are distributed mainly in the interfascicular planes, they appear to be present rather frequently in the vicinity of the smaller divisions of the coronary arteries which travel in these planes before ramifying as capillaries within the cardiac muscle fasciculae.

From an embryogenic standpoint, our observations indicate that "myocytes" arise predominantly as perivascular cells, inasmuch as they appear first just external to the endothelial lining of the primitive heart. It appears further that the "myocytes" permeate the entire myocardium in that stage of embryonal development of the heart in which the myocardium consists of a loose, trabecular spongework of muscle fibers, clothed by endocardial extensions and indippings. Further evidence of the derivation of these cells from the subendothelial layer of the endocardium is offered by the large number of "myocytes" present within the endocardial cushions, which represent the earlier stages in the formation of the cardiac valves.

Previous conceptions of the derivation of the "myocyte" from degenerated muscle cells were undoubtedly the result of histologic methods which were not adequate for the separate distinction of "myocyte" nuclei closely applied to muscle fibers, as well as of the fact that these cells were studied incidentally to observations carried out on areas of degenerated myocardial fibers. It is evident that had the hearts of embryos and the newborn of both animals and man been studied, in both of which "myocytes" are numerous and easy to find, the question of the origin of these cells from degenerating muscle would never have been even considered. Later observers like Watjen<sup>6</sup> and Semsroth and Pool,<sup>8</sup> in fact, gave up the idea of a myocardial origin because the former found typical "myocytes" in the epicardium in a case of rheumatic myocarditis, and the latter, at the base of vegetations on the mitral valves, in both of which areas cardiac muscle tissue is not present. Anitschkow's<sup>1</sup> discussion and illustration of intermediate stages in the transformation of myocardial fibers through degeneration into "myocytes" must therefore be revised on the basis of the observations reported by later investigators and those which we have recorded here.

Nevertheless, the observations of Saltykow,<sup>3</sup> Von Oppel,<sup>2</sup> Anitschkow<sup>1</sup> and others relating to the prominent appearance of "myocytes" in processes reactive to injury, inflammation or foreign body irritation

in the myocardium are correct. We also have observed proliferative and reactive changes in the "myocytes" in a number of other cardiac pathologic states. These include sepsis, meningococcic bacteremia, scarlet fever, periaarteritis nodosa and subacute bacterial endocarditis. Recently our attention has been drawn to a peculiar focal granuloma in the myocardium by Dr. Paul Klemperey, in which again the "myocytes" showed some proliferation. Anitschkow<sup>1</sup> and others have already observed that not only are "myocytes" present in the damaged region in significant numbers but their cytoplasm is prominent and distinctly basophilic in tone. These observations have been confirmed by us and appear to signify further a dedifferentiation to the embryonal stage, since it is precisely this prominence of the cytoplasm and its basophilic tone which characterize these cells in certain stages of their embryonal development, as described in a previous section of this paper. The observations of the authors quoted regarding the activity of the "myocytes" in reparative processes require much further study, especially since the distinctive appearance of "myocytes" makes it possible to trace with ease much of the morphologic alteration which they may undergo in disease processes. Jacki<sup>5</sup> and Watjen<sup>6</sup> noted the resemblance of the nuclei in certain cells which compose the Aschoff body to "myocytes," and Jaffe<sup>10</sup> also referred to this resemblance. One of us (J. C. E.) has made this observation also, and a separate paper devoted to this question is now in preparation. The resemblance of the "myocytes" to certain cells constituting the Aschoff body may well have played a part in the concept which has been prominent for some time that the Aschoff body is a derivative of necrobiotic cardiac muscle fibers.

Of the various sources which have been proposed theoretically for the origin of the "myocytes," namely, degenerating muscle, vascular endothelium, fibroblasts and histiocytes, only the last appears to us to be tenable on the basis of our own observations. Wenezianowa-Grusdkowa<sup>9</sup> ascribed a reticuloendothelial origin to these cells because of their ability to store intravital dyes. (Anitschkow<sup>1</sup> also mentioned the ability of these cells to function as phagocytes.) Jaffe<sup>10</sup> did not accept Wenezianowa-Grusdkowa's evidence of storage capacity as a proof that this cell is not derived from muscle, contending that muscle may also store intravital dye under certain conditions. Inasmuch, however, as the storage of dye in muscle requires tremendous overloading with dye, which was apparently not the case in Wenezianowa-Grusdkowa's experiments, we are inclined to accept the existing evidence that the "myocyte" may assume an active phagocytic function.

However, we do not agree with Wenezianowa-Grusdkowa's suggestion that the "myocytes" are derived from free or circulating elements of the reticuloendothelial system (polyblasts). Our observations indicate rather that these cells belong to the fixed elements of the reticulo-

endothelial system in the myocardial framework, as suggested by Jaffe, being derived originally from subendothelial or perivascular fixed cells. The active participation of the "myocyte" in the aforementioned reactive states of the myocardium, and no doubt in many others not yet investigated, is entirely consistent with the role played by the reticuloendothelial system in reparative processes.

It is evident from the foregoing discussion that fundamental objections exist to the continued use of the term "myocyte" for these cells. We feel that the characteristic appearance of this cell and its essential nature can be better expressed by the term "myocardial reticulocyte."

#### SUMMARY

The Anitschkow "myocyte" is a normal constituent of the human and vertebrate heart during the embryonal and postembryonal stages of development. This cell is part of the supporting tissue of the heart and belongs to the fixed elements of the reticuloendothelial system. It plays a definite role in inflammatory and other defense reactions. The name "myocardial reticulocyte" is suggested as a more accurate designation for this cell.

# CHANGES IN THE PROSTATE CAUSED BY HIGH FREQUENCY CURRENT

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The introduction and popularization of transurethral prostatic resection created a diagnostic problem in pathology which requires clarification particularly for the clinical urologist and those unfamiliar with pathologic diagnosis. The difficulty arises from the fact that the current produces a distortion of tissues which gives a confusing picture and makes the pathologic interpretation difficult.

The changes produced by transurethral resection are of two kinds. The first kind includes those found in the tissues removed with the resectoscope, these changes are produced at the moment of penetration of the tissue by the current. To the second kind belong those changes which are a reaction of the organism and which appear in the prostatic tissue remaining in the body, after a few hours or longer. The secondary changes are very important from the clinical point of view whereas the primary effect of the procedure on the tissues is interesting rather from the pathologic point of view.

The effect of high heat and various currents on other tissues has been examined by many authors. But we found only two publications dealing specifically with our subject. Caulk and Harris<sup>1</sup> published their microscopic observations in connection with operations on dogs and 2 human subjects. They mentioned in general the superficial necrosis and the shrinking of cells and nuclei in the material examined immediately after operation, but they did not go into details. They found also in the prostate removed a variable time after resection secondary necrobiotic changes. Smith and Stockwell<sup>2</sup> examined the effect of electrodehydration on prostatic resection. In 1 case they examined the prostate twelve hours after resection and in their microscopic examination found only the necrosis of the superficial tissue.

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From St Istvan Hospital, Budapest, Hungary, under the direction of Prof Odon Zalka

1 Caulk, J R, and Harris, W. J Urol **32** 473, 1933

2 Smith, C K, and Stockwell, L. J Urol **34** 31, 1935

and hemorrhagic infiltration significant. They did not find the secondary changes described by Caulk and Harris.

Our work consisted in the examination of pieces of prostatic tissue removed from 10 human subjects by transurethral resection and 40 prostates removed either from cadavers or from patients by prostatectomy, to which the usual resection procedure had been applied. Tube type and spark gap type machines were employed. The strength of current used was 4 to 10 amperes, the duration of the application of the current varied from two to eight seconds.

We found in the microscopic picture of the tissues obtained by transurethral resection conspicuous and characteristic features, the most confusing of which is the elongation of cells and nuclei to be described later. If this occurs in the epithelial cells it produces fantastic, distorted glands, which make a correct diagnosis difficult.

#### MICROSCOPIC OBSERVATIONS

Since we found only small quantitative differences between the various cases of transurethral resection, we summarize our observations. We distinguish in general three layers in the microscopic picture. The most superficial forms a very thin, brownish yellow, torn, charred edge. Below this is a vacuolated, spongy layer, which is already well known in cases of electric injuries of other nature and damage of tissues caused by heat. This corresponds to the picture called *Wabenhof* or *Hutzwaben* by the German authors. In general, this layer is also thin, but sometimes it can be followed between the loose connective tissue down to the deeper layers. The septums which separate the vacuoles are homogeneous, and the original structure cannot be recognized in them, only one or two nuclei, which stain very lightly. This layer stains with hematoxylin diffusely light blue (fig 1A).

The first and second layers are thin. The third layer is much more conspicuous and extensive, sometimes comprising the remaining part of the prostate, if the removed particles are small. In this layer the original tissue structure becomes indistinct, i. e., more or less homogeneous, especially in the firmer parts. Nevertheless it can be recognized. The fibers of the connective tissue become confluent, forming bundles which stain bluish with hematoxylin, and they lie close to the neighboring smooth muscle tissue cells. The latter also are confluent, forming hyalin-like islands between the light blue fibers of connective tissue, and they stain well with eosin. In the nuclei there can be observed also a vacuolated swelling. The walls of the small vessels also stain homogeneously light blue and form a ring lined with endo-



Fig 1—*A* shows the three layers (a) the charred edge, (b) the spongy layer and (c) the coagulation layer *B* shows the considerable elongation of the epithelial cells in the superficial layer

thelium In this layer the nuclei of the finer fibrous connective tissue are deformed and shrunken, or they appear as either wavy or straight threadlike formations and are in general hypochromatic The nuclei in the loose connective tissue around the vessels and glands are less shrunken and hyperchromatic

In this layer the epithelium of the glands shows significant changes, the essential one being the considerable elongation of the epithelial cells and their nuclei (fig 1*B*) The cells may become five or six times their original height The elongation of the epithelial cells may reach such an extent that the glandular lumens can hardly be recognized in many places In this way a picture may be seen which reminds one of solid islands The protoplasm stains lightly The place of the nucleus is sometimes taken by an elongated, threadlike formation The staining of the elongated nuclei is generally intensive, and in certain parts it shows segmentation The nuclei are generally parallel and are mostly straight or show one or two wavelike curvatures, the whole group of elongated nuclei showing a hair bundle-like picture (fig 2*A*) Such alveoli, which show elongation of their nuclei and are in consequence distorted, occur not only in the connective tissue which becomes homogeneous but sometimes also in the deeper layers of connective tissue which do not show any artificial changes

We performed the usual resection procedure on 40 prostates, removed either surgically or from cadavers, in order to examine whether the changes described are those of vital reaction or those merely of a passive physical nature The prostates were preserved in a refrigerator, and the current was applied one half to six days after death

We found on histologic examination changes similar to those found in the tissues removed from the human subject by transurethral resection, the differences being only quantitative (fig 2*B*) The parts affected are essentially smaller, being only one-half or one-third as wide, and the effect of the current on the individual tissue elements is also considerably smaller The elongation of the nuclei is much less, occurring only on the side of the gland which is close to the surface and showing a gradual decline toward the parts farther from the treated area In such glands the nuclei which are not elongated show only a hyperchromatic character When the resections have been done with different strengths of currents, there is no proportion between the severity of the changes and the intensity of the current The time elapsing between death or operative removal of the gland and application of current seemed to play no role in the degree of changes found microscopically



Fig 2—*A* shows the hair bundle-like picture presented by the elongated nuclei, *B* the nuclear elongation in a cadaver prostate five days after death



## COMMENT

Many authors have found changes in other organs and tissues caused by other agents similar to those found in transurethral prostatic resection. Especially conspicuous is the similarity of the characteristic elongation of the glandular epithelial cells found in the bronchial passages and in the gastrointestinal tract caused by heat (e. g., burning, inhalation of hot air, touching with red hot metal). As to the mechanism of the origin of the changes, opinions of the various authors differ. Some have stated that the effects of the current are due to heat, while others have found them to be the result of a specific electro-mechanic action. Pietrusky<sup>3</sup> and Schridde<sup>4</sup> stated that the electrolytic factor also plays a role. Many authors have found quite similar cell deformities due only to heat. In using electric current one cannot exclude its electrolytic and electrodynamic role, but we find Joule's heat effect the most important factor.

As to the question of whether or not the elongation of nuclei is of vital character, the result of our experiments on prostates removed from cadavers 5 to 6 days old convinces us that it is merely a physical phenomenon. The irregularity of the changes found in these prostates at varying periods after death and the quantitative differences of changes in the operative and cadaver material show that the degree of the effect of the resection procedure depends very much on the moisture content of the tissue. An observation of ours made on a prostate five days after removal speaks in favor of this supposition. This prostate was preserved in a closed bottle for five days, and we made the resection on the side which was in contact with the wall of the glass. In consequence of this, the loss of moisture was minimal. In this case we found a greater amount of nuclear elongation than in some specimens only 1 or 2 days old.

## SUMMARY

Tissue particles obtained by transurethral prostatic resection show considerable distortion. The changes consist of three layers of different structure: a charred thin superficial layer, a spongy coagulative layer of varying width and a wider layer which becomes homogeneous in varying degrees.

The alveolar epithelium becomes much elongated, threadlike and packed in close bundles. Knowledge of the nuclear elongation is important because it may lead to diagnostic mistakes. We were able to produce similar but smaller changes in cadaver prostates five to six days after death. The degree of the changes produced seems to be independent of the strength of the current but seems to be dependent on the moisture content of the tissue.

<sup>3</sup> Pietrusky, F. *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **6**: 535, 1926.

<sup>4</sup> Schridde, H. *Virchows Arch. f. path. Anat.* **252**: 774, 1924.

# MEDIAL DEGENERATION OF THE AORTA

A STUDY OF TWO HUNDRED AND TEN ROUTINE AUTOPSY SPECIMENS  
BY A SERIAL BLOCK METHOD

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It is proposed in this paper to present a survey of medial degeneration of the aorta as encountered in routine autopsy specimens. Weise<sup>1</sup> alone attempted a similar study. Others who described medial degeneration in the intact aorta studied selected<sup>2</sup> or isolated specimens<sup>3</sup>. The majority of workers write of medial degeneration in spontaneously ruptured aortas<sup>4</sup>. It must be mentioned that Moritz<sup>5</sup> also investigated the intact aorta, but his purpose was essentially to note the distribution of chromatotropic substances in the media.

## MATERIAL

The material used in this study consists of 210 aortas from persons in all decades of life (table), who died from a variety of conditions. No attempt was made to select specimens, in order to insure as varied a group of diseases as possible. The large number precludes enumeration. However, persons from whom the specimens came died of such conditions as carcinoma, acute infections, heart disease of various types, primary blood dyscrasia (leukemia, pernicious anemia, agranulocytosis, aplastic anemia and others), cirrhosis of the liver, bleeding and perforated ulcers of the stomach, diabetes, trauma, gas poisoning, postpartum hemorrhage, Addison's disease, acute hemorrhagic pancreatitis and so on.

## METHOD OF PREPARATION

The entire ascending aorta and arch were cut into serial transverse blocks, six to ten in number, 3 to 5 mm in length, each block including the entire circumference of the vessel. In addition, longitudinal strips were selected from the thoracic aorta, two to four in number. From each block two sections were prepared, of which one was prepared with the hematoxylin-eosin stain and the other with Weigert's elastic tissue stain. In 150 cases a map of the aorta was made, on which was indicated the level of each section studied. This was done

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1 Weise, W. Beitr. z. path. Anat. u. z. allg. Path. **93** 238, 1934

2 Cellina, M. Virchows Arch. f. path. Anat. **280** 65, 1931

3 Rottino, A. Arch. Path. **27** 320, 1939. Harrison, F. F. *ibid.* **27** 742, 1939

4 (a) Gsell, O. Virchows Arch. f. path. Anat. **270** 1, 1928. (b) Erdheim, J. *ibid.* **273** 454, 1929, **276** 187, 1930

5 Moritz, A. R. Am. J. Path. **8** 717, 1932

to make possible an accurate survey of the distribution of the lesion, especially as to its presence or absence in the descending portion of the vessel

#### OBSERVATIONS

Degenerative changes beneath atherosclerotic plaques were not considered in this study. Despite this limitation, lesions were found in 95 aortas. The most common lesion, present in 92 aortas, was similar to one described in a previous communication<sup>6</sup> as simple muscle loss. It was characterized by loss of muscle cells from focal areas of media. Though the continuity of the elastic lamellae in these areas remained intact, the lamellae became crowded so as to narrow the interlamellar spaces (fig 1 *A* and *B*). Anuclear muscle cell bodies such as those described by Gsell<sup>41</sup> were not recognized. In 5 of the 92 aortas there were additional areas in which the elastic elements were disintegrated or in the process of disintegration, leaving defects which were occupied chiefly by a basophilic mucoid fluid (fig 2 *A*) and in 1 specimen by a loose fibrillar connective tissue (fig 2 *B*). In another aorta—the ninety-third—there were numerous irregular narrow foci in which elastic lamellae were absent while muscle cells remained (fig 3 *A* and *B*). The latter were conspicuous in that their longitudinal axes lay perpendicular or oblique to those in adjacent normal areas. Cystic spaces filled with mucoid fluid were also present in this aorta, but nowhere were areas of simple muscle loss found. In the final 2 specimens the sole change consisted of cystic areas filled with mucoid fluid.

The lesions were distributed principally in the ascending aorta and arch. In 5 aortas they were found, in addition, in the descending thoracic portion and once only in the abdominal portion. Within the media the lesion was chiefly located in the middle, not infrequently in the inner and rarely in the outer third.

Based on the number and size of the lesions, the severity of the process was graded as 1 plus (50 cases), 2 plus (15 cases), 3 plus (6 cases), and 4 plus (24 cases). The 1 plus lesion was insignificant, one to three small areas of involvement being found in the entire aorta. In the 4 plus group as many as two to six lesions were found in every section. Further they were large, involving as much as one quarter to one half of the thickness of the media. The lesions of 2 plus and 3 plus grades occupied intermediate positions of severity. In none of the cases was a cellular reaction present in or adjacent to the lesions described. No relationship was encountered between the presence of the lesion and changes either in the vasa vasorum or in the adventitia.

#### ANALYSIS OF OBSERVATIONS

In an earlier published report<sup>6</sup> on medial degeneration as encountered in the ruptured aorta, advanced age, hypertension and some

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6 Rottino, A. Arch Path 28 1, 1939

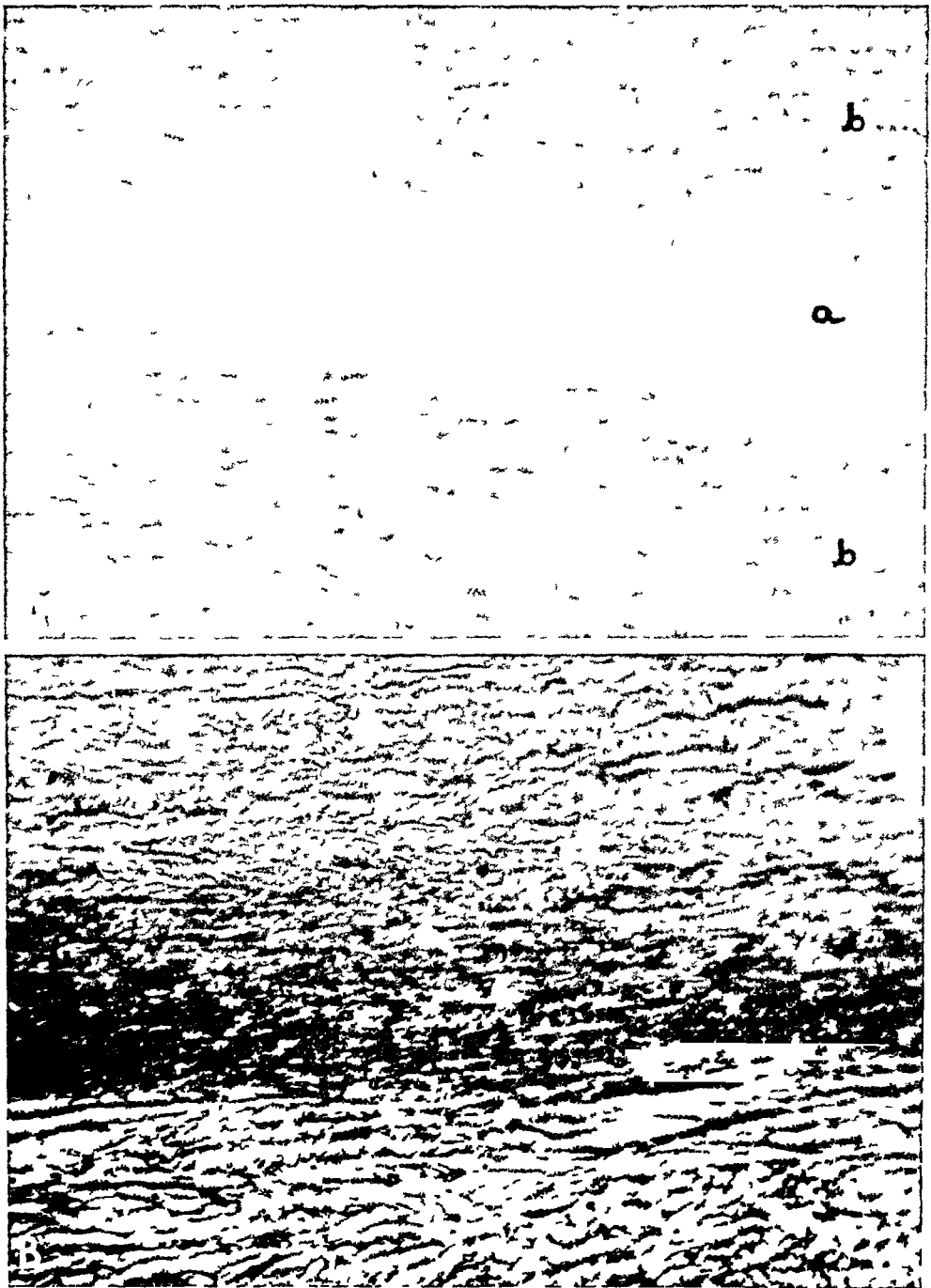


Fig 1—*A*, hematoxylin-eosin stain showing, under low power magnification, (*a*) an area of simple loss of muscle and (*b*) normal media. *B*, Weigert's elastic tissue stain showing, under low power magnification, crowding of elastic laminas and narrowing of interlamellar spaces in an area of simple loss of muscle. In adjacent areas the lamellae are properly spaced.

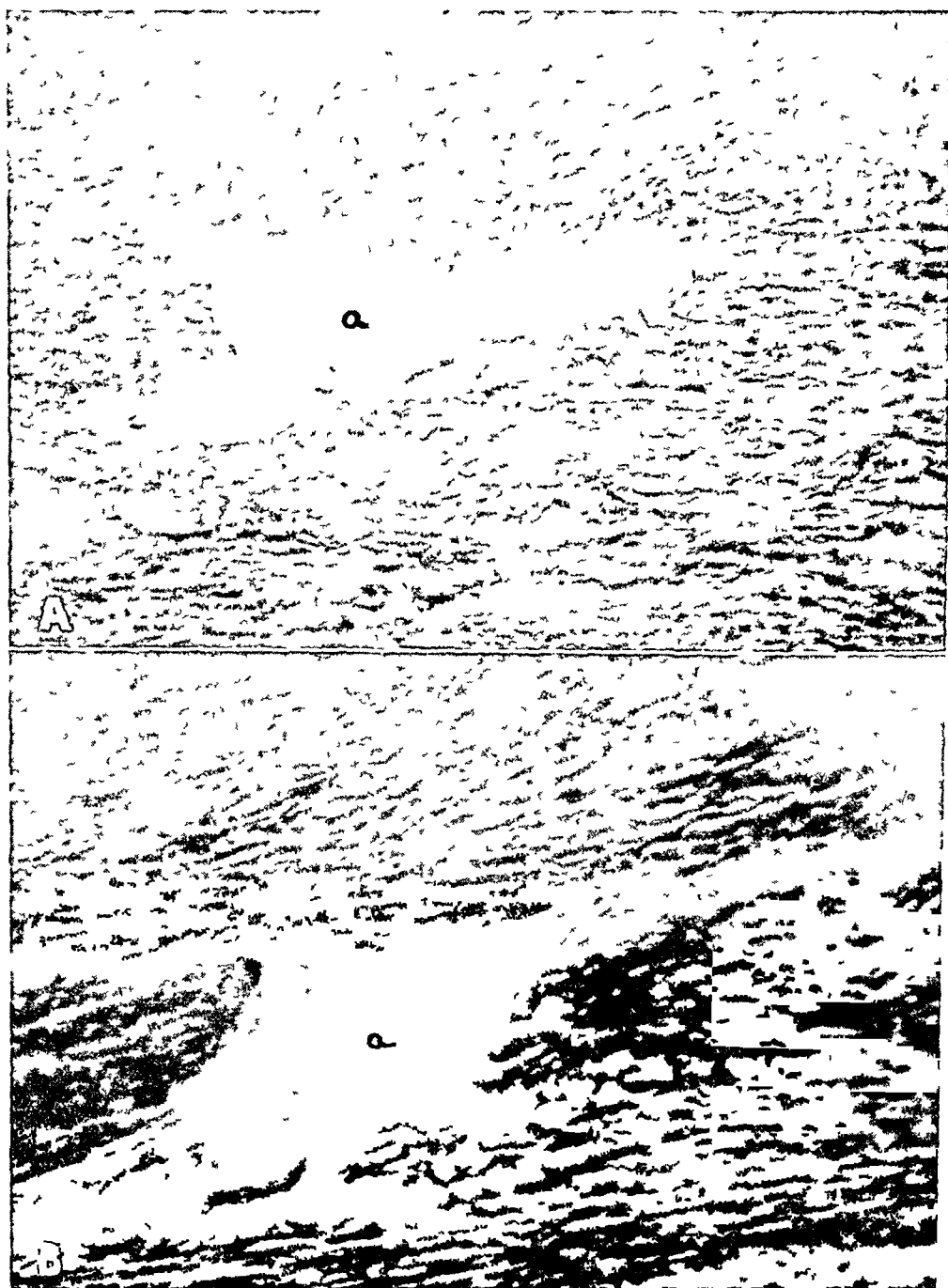


Fig 2—*A*, Weigert's elastic tissue stain showing, under low magnification, a cyst (*a*) in the media which with the hematoxylin-eosin stain is seen to contain a basophilic substance. *B*, Weigert's elastic tissue stain showing, under low power magnification, a defect (*a*) in the media. The substance occupying it is largely a loose fibrillar tissue, probably collagen. A basophilic material is present in the interstices of the fibrils.

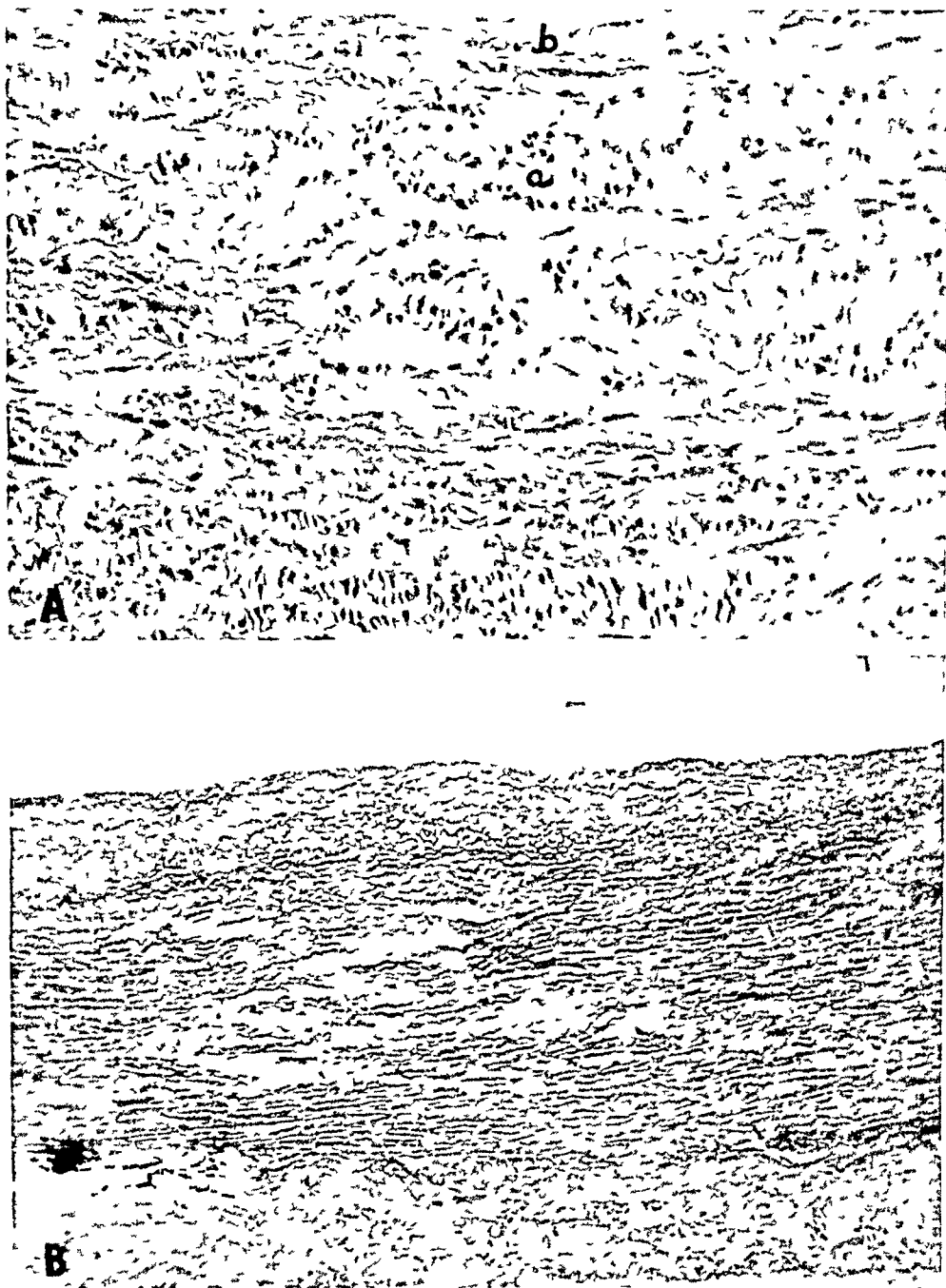


Fig 3—*A*, hematoxylin-eosin stain showing, low power magnification, a medial defect occupied by muscle (*a*) the long axis of which lie oblique and perpendicular to that of muscle in normal adjacent media (*b*) *B*, Weigert's elastic tissue stain showing, low power magnification, loss of elastic laminas in medial defects similar to that shown in *A*

form of organic heart disease were noted to be present sufficiently often to be considered significant. Mindful of this, in the present study I compared the 95 cases in which medial degeneration was present with the remaining 115 in which this lesion was absent.

From the table it will be seen that the lesion is less common in persons below 40 years of age (in 10 of 44, or 20 per cent) than in those beyond 40 (in 85 of 166, or 50 per cent). It was present in 17 of 25 persons beyond 70 years of age. Further, in the younger group significant lesions (2 to 4 plus) occurred in 3 of 10 persons, whereas in the older group such lesions occurred in 40 of 85. Hence it would appear that medial lesions are encountered more often and are of a severer grade in the advanced age group.

*Medial Degeneration of Aorta*

Age, yr	Aortas	Medial Degeneration of Aortas		Aortas in Which Medial Degeneration Was of Given Grade of Severity			
		Absent	Present	+	++	+++	++++
5-10	3	3	0	0	0	0	0
11-20	9	7	2	2	0	0	0
21-30	8	8	0	0	0	0	0
31-40	24	16	8	5	1	0	2
41-50	46	29	17	10	1	2	4
51-60	46	23	23	13	5	1	4
61-70	48	20	28	15	5	2	6
71-80	25	8	17	5	3	1	8
81-85	1	1	0	0	0	0	0
Total	210	115	95	50	15	6	24

To evaluate the age factor, uncomplicated by hypertension and organic heart disease, those cases were selected in which both of these conditions were absent.<sup>7</sup>

There were 67 such cases. Medial degeneration occurred in 23 of them (34 per cent). Of these patients 16 (70 per cent) were above 40 years of age. In 19 of the 23 cases the lesions were only of 1 plus severity. Thus it seems that age continues to be a factor despite the fact that the incidence of lesions and the degree of severity are less.

The effect of rheumatic heart disease and that of hypertension were next studied in 23 and 54 cases, respectively. With regard to rheumatic heart disease, the cases comprising the group studied were those in which gross involvement of one or more valves of varying degrees of severity was shown. Cases in which the process was active and cases in which the disease was inactive were included. In every case gross

<sup>7</sup> The cases excluded were those in which there were questionable lesions of the valves, auricular or ventricular endocarditis, pericarditis either recent or old, cardiac enlargement, coronary disease, myocardial fibrosis as determined by gross examination and the study of several microscopic slides, or any evidence of syphilis of the aorta.

observations were substantiated by microscopic examination of one or all valves, mural endocardium, pericardium and myocardium. In 47 of the cases in which hypertension was recorded this was of the essential type, and the usual gross and microscopic changes associated with such a condition were present. The causes of death were chiefly heart failure, cerebral hemorrhage and uremia. In the remaining cases death occurred from miscellaneous causes. Five cases in which blood pressure was unrecorded or was normal or low were included because in these hypertrophy of the heart, nephrosclerosis and arteriolar changes were presented. The patients died either of coronary occlusion with myocardial infarction or from massive cerebral hemorrhage. One additional case was included in which the blood pressure was normal during the patient's stay in the hospital but in which there was a reliable history of repeated periods of hypertension. The final case included in the series was one in which high blood pressure was related to chronic diffuse glomerulonephritis. Cases in which there were unrecorded hypertension and enlargement of the heart but in which arteriolar and renal changes were lacking were not included, so as to escape the criticism of failing to recognize idiopathic hypertrophy. Cases in which hypertension was recorded without concomitant cardiovascular changes were also excluded, since the elevation of blood pressure was not rechecked and in most instances could be ascribed to a terminal cerebral accident.

The effects of other types of heart disease were not studied because the numbers of cases were too few.

There was no indication that the presence of rheumatic heart disease increases the incidence of medial degeneration beyond that of the non-cardiac group. The incidence of medial degeneration in the rheumatic group was 31 per cent (7 of 23 cases). Five of the patients were above 40 years of age. The lesion was of from 2 to 4 plus severity in 3 cases. In the hypertensive group, however, the incidence rises to 51 per cent. Further, there is an increase in the number of cases in which severer grades of medial degeneration were present (17 of 28), with 2 to 4 plus lesions. All patients in this group were above 40 years of age. Unfortunately, there were not, in the hypertensive group, enough patients below 40 years of age to permit study of the effect of elevated blood pressure on such a group.

#### COMMENT

The lesion of simple muscle loss noted in this study was similar to the medionecrosis observed by Gsell<sup>4a</sup> in spontaneous rupture of the aorta with the exception that necrotic muscle cells were not demonstrable. Cellina,<sup>2</sup> Weise<sup>1</sup> and Moritz<sup>5</sup> describe simple muscle loss in the intact aorta—the first author, in 9 of 10 selected aortas from persons between



71 and 95 years of age, the second, in 9 of 120 specimens unselected in regard to age and the last author in 6 of 70. The increased incidence in the present series is due probably to the use of large sections. Whereas Weise<sup>1</sup> and Cellina<sup>2</sup> in their cases both failed to observe more advanced lesions, in my study muscle loss, elastic tissue disintegration and cyst formation were seen in 8 aortas, and a lesion apparently similar to muscle regeneration in the sense of Erdheim was seen in 1 aorta.<sup>8</sup> Scars of the type described by Gsell were noted once.

Inasmuch as medial degeneration produced no macroscopically detectable changes or clinically demonstrable symptoms, its importance in the cases studied must be considered as nil. Despite this, its occasional significance in dissecting aneurysms must not be lost sight of, the latter catastrophe occurring most likely from extensive medial degeneration affecting strategic areas. Its further significance is attested by the fact that in occasional instances changes visible to the naked eye are observed, such as thinning of the wall, replacement of the yellow media by gray scars and finally wrinkling of the intima, suggesting syphilis (Erdheim, 1929)<sup>4b</sup>. In 1 case the change led to aneurysmal dilatation.<sup>3</sup>

Much has been written on the causes of medionecrosis. The importance of age is noted by Cellina,<sup>2</sup> for he found medial degeneration in 9 of 10 cases in which all the patients were older than 72 years. The high incidence in the aged is corroborated in the present study. Medial degeneration has been described in aortas from patients with rheumatic fever.<sup>9</sup> It was likewise observed in aortas from persons with rheumatic fever in the present series. However, there was no increase in incidence over that of a control group. It is fully realized, however, that the number of specimens from persons with rheumatic fever in this series is too small to enable one to draw hard and fast conclusions.

The greater frequency of medial degeneration in a group with hypertension gives the latter state etiologic significance. The mechanism can only be conjectured. It may be exhaustion of the contracting muscle in its effort to prevent overdilatation of the aorta. It may be the sudden tug on the wall of the vessel caused by abrupt increases in pressure in a vessel already the seat of hypertension. Finally, Erdheim (1930)<sup>4b</sup> offered an explanation on the basis of hypertepinephrinism, a substance which he suggests may be present in excessive amounts in persons with hypertension. This by causing spasm of the vasa vasorum, may lead to focal ischemia and necrosis.

8 That the picture seen in this lesion represents muscle regeneration was questioned by Cellina (Cellina, M. *Arch ital di anat e istol pat* **2** 1105, 1931). He felt instead that it is due to a clumping of the original muscle following loss of support through disintegration of elastic tissue.

9 Klotz, O, and Simpson, W. *Am J M Sc* **184** 455, 1932. Pappenheimer, A. M., and Von Glahn, W. C. *ibid* **44** 489, 1924.

## SUMMARY

Medial degeneration was noted in 95 of 210 aortas from routine autopsy material. In 92 of these the lesion was a simple loss of muscle. In 8 aortas only were advanced changes seen, such as loss of elastic tissue and cyst formation.

In 67 vessels from persons without organic heart disease or hypertension the incidence of medial degeneration was 34 per cent. The lesions were of mild degree. Seventy per cent of the patients were above 40 years of age.

In 23 aortas from patients with rheumatic heart disease the incidence of medial degeneration was 31 per cent. In 54 aortas from patients with hypertension the incidence rose to 51 per cent. Also the number with severe grades was greater.

# Case Reports

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## MULTINUCLEATE EPITHELIAL GIANT CELLS WITH INCLUSION BODIES IN PRODROMAL MEASLES

### Report of an Autopsy

K H SAMSROTH, M D, AMSTERDAM, N Y

Amitotic giant cells form a characteristic response of the host cells to some of the virus diseases, as pointed out by Rivers<sup>1</sup>. In the case of measles, a disease generally believed to be due to a filtrable virus, multinucleate giant cells have been found in the lymphatic organs. Since Warthin,<sup>2</sup> in 1931, pointed out the pathognomonic nature of these cells, they have been observed<sup>3</sup> as early as five days prior to and<sup>3c</sup> as late as two days after the appearance of the exanthem. Lesions of the respiratory tract, equally characteristic for measles, had not been described until Masugi,<sup>4</sup> in 1938, reported on the occurrence of epithelial multinucleate giant cells with cytoplasmic inclusion bodies in the pharynx, trachea and bronchi four days after the appearance of the exanthem. In prodromal measles, pathognomonic changes other than those of the lymphatic organs have as yet not been described. Such lesions, observed in the respiratory tract as well as in the lymphatic apparatus prior to the outbreak of the exanthem, are the subject of the present report. Permission to use the clinical records of this case was given by Dr S L Homrighouse, of Amsterdam, N Y.

### REPORT OF A CASE

A boy 20 months old was twice exposed to measles in the course of two weeks. About fourteen days after the first exposure he began to suffer from acute, severe dyspnea and died of respiratory difficulty two hours after an unsuccessful attempt at bronchoscopic examination. At autopsy three small erosions of the laryngeal mucosa were seen besides edema and congestion of the trachea and large bronchi. With these extremely scanty gross changes, the case remained unexplained until sections of the respiratory tract and lymphatic organs revealed lesions indicative of measles.

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From the Montgomery County Laboratories, Amsterdam, N Y, and the Department of Pathology, Albany Medical College, Albany, N Y.

1 Rivers, T M. *Am J Path* **4** 91, 1938.

2 Warthin, A S. *Arch Path* **11** 864, 1931.

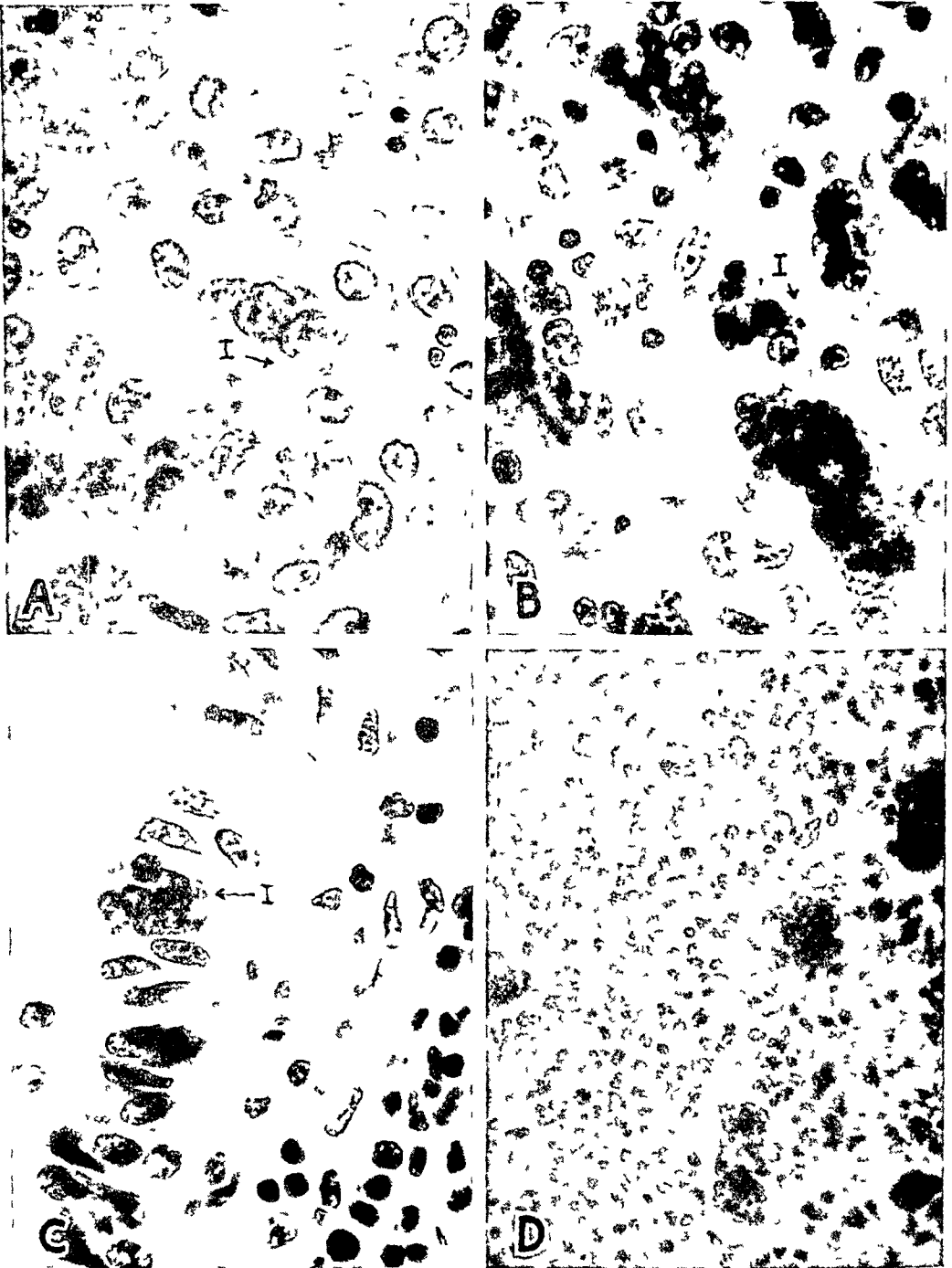
3 (a) Finkeldey, W. *Virchows Arch f path Anat* **281** 323, 1931, (b) **284** 518, 1932. (c) Davidsohn, J, and Mora, J M. *Arch Path* **14** 757, 1932. (d) Herzberg, M. *J A M A* **98** 139, 1932. (e) Fischer, W. *Beitr z path Anat u z allg Path* **91** 474, 1933. (f) Schultze, W H. *Munchen med Wchnschr* **80** 576, 1933. (g) Hathaway, B M. *Arch Path* **19** 819, 1935. (h) Graeff, S. *Deutsche med Wchnschr* **63** 1357, 1937.

4 Masugi, M, and Minami, G. *Beitr z path Anat u z allg Path* **101** 483, 1938.

Retrogressive epithelial changes were seen at the base of the tongue and throughout the respiratory passages. In all of these locations vacuolation, occasional pyknosis and desquamation of epithelial cells were present. Lymphocytes and, in lesser number, polymorphonuclear leukocytes were migrating through the epithelium. In the larynx and trachea the degenerative epithelial changes had progressed to the formation of erosions. In the epithelium of the tongue one found an occasional multinucleate giant cell, with a cluster of three or four centrally situated nuclei (*A* in figure). In some instances there was contained in the cytoplasm a group of faintly basophilic globoid bodies. No mitoses were seen. Many such amitotic giant cells were seen in the epithelium of the tonsillar crypts (*B* in figure). Small globoid inclusion bodies occurred in their cytoplasm. In this location they were usually eosinophilic. The oral mucosa and the mucosa of the entire upper respiratory tract were diffusely infiltrated with many mononuclear round cells and few polymorphonuclear leukocytes. Plasma cells predominated around the mucous glands of the trachea and in the larynx. Some of them contained two or three nuclei, others, a cluster of five or more, surrounded by somewhat ill defined cytoplasm—a picture suggesting the formation of multinucleate giant cells through amitotic division of the nuclei of plasma cells.

The epithelium of the bronchi showed vacuolation and desquamation, with complete loss of the surface epithelium in places, particularly over lymphoid nodules. An outstanding feature was the presence of multinucleate giant cells in the epithelium (*C* in figure). A cluster of six or more hyperchromatic nuclei could be seen in an epithelial cell, which might or might not retain its cilia. Some of the giant cells contained eosinophilic inclusion bodies, usually globoid or oval. As a rule, they were situated at the basal side of the cluster of nuclei. They were not seen in other epithelial cells. In some giant cells fusion of nuclei had led to the formation of large polylobate masses of chromatin, found within the epithelium or on its surface. The epithelium of the bronchial glands also showed an occasional multinucleate giant cell. No mitoses were seen. Mononuclear round cells, few polymorphonuclear leukocytes, plasma cells and multinucleate plasma cells infiltrated the bronchial mucosa. The lungs proper showed peribronchiolitis, with predominance of mononuclear round cells. Adjoining interalveolar septums were frequently thickened through an increase in the number of their nuclei. Occasionally, large polylobate masses of chromatin were seen in alveolar lumens. Some of them showed cytoplasm provided with cilia, obviously aspirated bronchial epithelium. Here and there a cell of the alveolar lining showed a cluster of hyperchromatic nuclei. Interlobular edema was marked.

Of the lymphatic organs, the palatine tonsils, peribronchial and hilar lymph nodes, spleen and thymus were examined. The tonsils were the seat of inflammatory hyperplasia. The germinal centers showed retrogressive changes. Within them numbers of multinucleate giant cells were present (*D* in figure). Their densely crowded hyperchromatic nuclei—up to twelve or more—left but a narrow margin of cytoplasm. The same type of giant cell was present in peribronchial and hilar lymph nodes, with lymphoid hyperplasia and retrogressive changes of germinal centers. It was also seen in many lymph follicles of the spleen and was apt to be located near the periphery of the germinal center. Lymphocytes and few eosinophilic round cells infiltrated the stroma of the thymus. Its parenchyma contained large numbers of multinucleate giant cells similar to those found in the tonsils and lymph nodes. The number of their small hyperchromatic nuclei varied from three or four to fifty or more. Within the largest ones fusion of



*A*, epithelium at the base of the tongue. At *I* is seen a multinucleate epithelial cell with cytoplasmic inclusion bodies. *B*, multinucleate giant cell with inclusion bodies (*I*) in epithelium of the palatine tonsils. *C*, multinucleate giant cell in epithelium of a large bronchus. At *I* an eosinophilic inclusion body is seen, surrounded by a clear zone. *D* palatine tonsil showing multinucleate giant cells in germinal centers of the lymphatic tissue.

nuclei into bizarre-shaped masses of chromatin had frequently occurred. No mitoses were found in thymus, tonsils or lymph nodes. Extensive phagocytosis of nuclear fragments by reticulum cells was observed.

#### SUMMARY

In a fatal case of prodromal measles with multinucleate giant cells in the lymphatic organs and thymus, certain lesions of the respiratory tract were observed which were considered characteristic. In the epithelium of the respiratory passages, degenerative changes were associated with proliferative ones. The latter consisted in the appearance of amitotic multinucleate epithelial cells comprising cytoplasmic inclusion bodies. In the underlying mucosa, amitotic division of plasma cells accounted for the formation of some multinucleate giant cells.

## PAPILLARY TUMOR OF THE CHOROID PLEXUS IN A NEWBORN INFANT

GERHART A. DRUCKER, M.D., BROOKLYN

In recent years the number of reported cases of papilloma of the choroid plexus has considerably increased. However, this type of tumor is still regarded by most authors as one of the rarest among all types of cerebral neoplasms. Cushing's<sup>1</sup> series of more than 2,000 verified cases of intracranial tumor contains 12 of papilloma of the choroid plexus, an incidence of 0.6 per cent. Van Wagenen,<sup>2</sup> who summarized the literature on this subject in 1930, collected 45 cases from the literature and added 2 of his own. In 1936 Friedman and Solomon<sup>3</sup> published a comprehensive paper on cases of tumor of the choroid plexus in childhood. Reviewing the previous literature, they were able to find 34 true cases in all age groups, including 1 case of their own. In 14 of these cases the patient had been an infant or a child. Another review of this subject, published by Turner and Simon<sup>4</sup> in 1937, mentions 72 reported cases. Since the publication of the latter review, several new case reports have appeared in the literature.

Papillary tumor of the choroid plexus has been found in all age groups, but the incidence is highest in infants and children. The youngest patient in all previously reported cases was a boy 10 weeks of age.<sup>5</sup> The case presented in this paper is remarkable because a papilloma of the choroid plexus was found at autopsy in a newborn infant.

### REPORT OF A CASE

Mrs. A. M., aged 28, was admitted to the maternity service of the Israel Zion Hospital on Feb. 14, 1938, in active labor. This pregnancy was her second. The first child was 6 years of age, normally developed and in good health. The family history was entirely irrelevant. A diagnosis of frank breech presentation was made, and the child was delivered by breech extraction. It was deeply cyanotic and showed no sign of life. The usual measures of resuscitation were applied but were not successful.

*Postmortem Examination*—The body was that of a full term newborn boy measuring 54 cm. in length and weighing 3,530 Gm. The skull was larger than is usual, the circumference of the suboccipitobregmatic plane measured 36.5 cm., that of the mentooccipital plane, 44 cm. Both fontanels were bulging slightly. The greater fontanel admitted the tips of two fingers, the lesser, the tip of one

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From the Department of Pathology of the Israel Zion Hospital.

1 Cushing, H. Intracranial Tumors, Springfield, Ill., Charles C. Thomas, Publisher, 1932.

2 Van Wagenen, W. P. Arch. Surg. **20** 199, 1930.

3 Friedman, J. J., and Solomon, C. I. Am. J. Dis. Child. **52** 114, 1936.

4 Turner, O. A., and Simon, M. A. Am. J. Cancer **30** 289, 1937.

5 Weinstein, E. A. J. Mt. Sinai Hosp. **5** 573, 1938.

finger. The cranial bones touched each other at the sutures. The face was deeply cyanotic, and the eyelids were edematous. All signs of maturity were present.

The scalp was markedly edematous. On opening the cranial cavity, there escaped approximately 100 cc of nonclotted blood. One noted a complete tear of the right half of the tentorium cerebelli and a partial tear of the vena cerebri magna. A large amount of loosely clotted blood was present in the subdural space and below the tentorium.

The brain measured 15 by 13 by 9.5 cm and weighed 310 Gm. The leptomeninges in large areas were hemorrhagic. There was evident at once marked atrophy of the cortex of the left parietal and occipital lobes, which showed no convolutions and no sulci. Palpation of this area revealed great flabbiness of the brain tissue, beneath which however, a mass of firmer consistency was felt. Removal of a thin shell of brain tissue in this area exposed a well outlined ovoid tumor, which measured 4 by 3.5 by 3 cm (fig 1). It was located within the greatly dilated posterior horn of the left lateral ventricle and was firmly attached



Fig 1—Lateral view of the left cerebral hemisphere. The thinned out cortex of the parietal and occipital lobes has been removed and the tumor within the greatly dilated posterior horn of the left lateral ventricle is exposed.

to its floor, medial wall and roof. The choroid plexus, coming from the inferior horn, entered the tumor at the junction of the inferior and posterior horns. Two large blood vessels, coming from the plexus, entered the tumor at this place. Further dissection showed that the wider of these two vessels was a direct continuation of the greatly dilated anterior choroid artery. The tumor was lined by a very thin, delicate, wrinkled fibrous capsule, rich in blood vessels near the junction of the choroid plexus and the growth. The surface of the growth was pale yellowish gray and showed deep furrows, along which the capsule was firmly attached to the underlying tumor tissue. In other places, particularly where the growth was fixed to the walls of the ventricle, a free space remained between the tumor tissue and its capsule. In these areas the capsule, on superficial examination, seemed to merge with the ependyma of the ventricle.

The cut surface of the neoplasm appeared papillary, soft and grayish pink. Many small papillae also originated from the inner lining of the fibrous capsule.



The cortex of the left parietal and occipital lobes and the underlying white matter were compressed to a shell 0.5 cm in thickness. Similar compression and atrophy, with loss of the normal convolutions, were seen on the medial aspect of the left occipital lobe. The anterior and inferior horns and the central part of the left lateral ventricle were normal in width. The optic thalamus was in front of the tumor and not compressed by it. The lentiform nucleus had no relation to the tumor. The remaining portions of the ventricular system were normal in width and lined by smooth, pale ependyma. The foramina of Monro, Magendie and Luschka were patent.

The organs of the neck showed nothing unusual. The lungs were strikingly emphysematous, undoubtedly as a consequence of the mouth to mouth breathing which had been done as a last effort of resuscitation. Along the edges of both

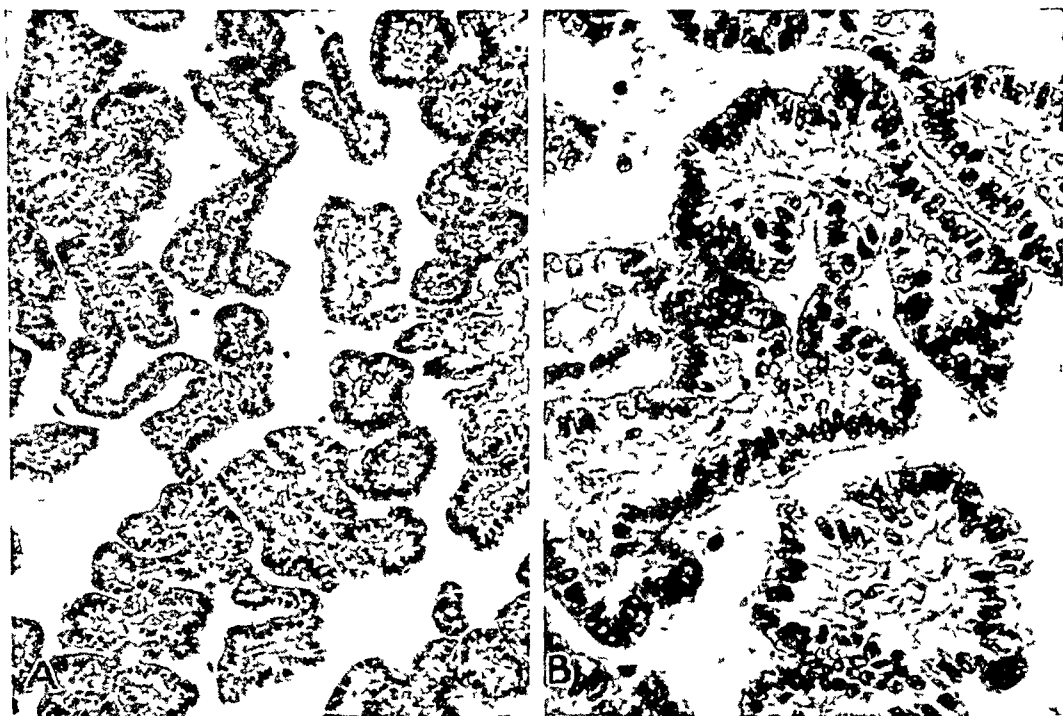


Fig 2—Section of papilloma of the choroid plexus, hematoxylin and eosin stain A, low magnification, B, high magnification

lungs were many thin-walled emphysematous blebs, some of them 0.5 cm in diameter. The pleura was smooth and shiny throughout. There were many subpleural petechial hemorrhages. The free and the cut surfaces of both lungs were bright pink, with only a few small dark areas of atelectasis interspersed. The heart was normal save for numerous subepicardial petechial hemorrhages, 1 to 2 mm in diameter. The remaining organs showed nothing unusual.

*Microscopic Examination*—Sections of the tumor showed an architecture of innumerable delicate dendritically branching papillae, each consisting of a core of vascularized connective tissue and lined by a single layer of tall columnar epithelium (fig 2A). The papillae originated from a scaffolding of dense connective tissue, which in some places formed cystlike spaces enclosing a great number of tumorous papillae. The lining epithelium showed a regular palisade-like arrangement. Each epithelial cell contained a round or spindle-shaped, moderately

hyperchromatic nucleus with numerous fine chromatin granules. In most areas each nucleus was in the peripheral half of the epithelial cell, leaving only a narrow strip of cytoplasm between its peripheral pole and the free surface of the cell. In other places the nucleus occupied the center or the basal portion of the epithelial cell. The cytoplasm of the epithelial cells was eosinophilic and granular in its peripheral half, whereas its basal portion was clear, showing the cell border distinctly. On their free surface many of the epithelial cells carried short delicate cilia, among which sometimes one solitary long cilium was seen. A real brush border was present in very few places. Blepharoplasts were absent in all sections.

The stroma of the papillae consisted of rather loose, wavy connective tissue which stained blue with Mallory's aniline blue-orange gold stain<sup>5a</sup>. The larger stromal septums contained many thin-walled arterioles and capillaries. Loosely scattered in between were fibroblasts, occasional lymphocytes and, in moderate numbers large clear mesenchymal cells. The smallest papillae consisted only of two layers of epithelium, sometimes arranged around a wide capillary. A colloid-like degeneration of the stroma was seen in many places, these areas stained bright red with Mallory's aniline blue-orange gold stain. Some of these degenerated papillae were markedly swollen and lined by cuboidal epithelium.

The capsule of the tumor consisted of a very thin layer of collagenous connective tissue, lined on the inside by papilliferous epithelium of the same type as that composing the tumor tissue itself. In many places bizarre papillomatous formations arising from the inner outline of the capsule protruded into the tumor tissue. Sections of those areas where the capsule was adherent to the ventricular wall showed an irregular furrow-like protrusion of the capsule into the surrounding brain tissue. In some places the capsular epithelium covered the underlying brain tissue so closely as to resemble an ependymal lining. By proper staining methods, however, the collagenous connective tissue of the capsule could be clearly demonstrated everywhere as a thin layer between the epithelium and the underlying brain tissue, proving beyond doubt that the former originated from the choroid plexus and not from the ependyma. Mitotic figures and atypical cell forms were absent in all sections.

Sections of the lungs revealed full aeration and marked emphysematous distention of all alveoli. The other organs showed no important histologic changes.

*Diagnosis*—The diagnosis was papilloma of the choroid plexus, located in the posterior horn of the left lateral ventricle of the brain, atrophy of the left parietal and occipital lobe, tear of the tentorium cerebelli and vena cerebri magna with massive subtentorial hemorrhage, acute diffuse pulmonary emphysema (the latter probably due to the attempts at resuscitation, including mouth to mouth breathing), multiple subpleural and subepicardial petechial hemorrhages.

#### COMMENT

This is the first reported case of papilloma of the choroid plexus in a newborn infant. However, several cases have been reported in which such a tumor was found in very young infants, and one is justified in assuming that in these cases the growth had been present at birth. This is particularly true of those cases in which an enlargement of the head was noted at birth or a few weeks afterward. In the case presented,

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<sup>5a</sup> Mallory, F. B. Pathological Technique. A Practical Manual for Workers in Pathological Histology, Philadelphia, W. B. Saunders Company, 1938, p. 153.

death was not caused directly by the tumor itself, but by a birth injury inflicted during the delivery of the aftercoming head. On the other hand, the tumor must be regarded as the indirect cause of death, since the enlargement of the head caused by the growth certainly predisposed to a tear of the tentorium, especially in breech presentation. Had the child survived its birth, neurologic symptoms would probably have been present from the very onset, in view of the size of the tumor and of the amount of compression of the adjacent structures that was already present.

Microscopically, the neoplasm showed all the characteristics of a papilloma of the choroid plexus. The absence of atypical changes and mitotic figures in the lining epithelium let it appear as a benign growth. On the other hand, an irregular protrusion of the capsular epithelium into the surrounding brain tissue was seen in several places, which seems to speak for an invasive character of the growth.

Since the tumor was adherent to the wall of the ventricle and since in some of these areas the papilliferous epithelium formed a lining of the underlying brain tissue resembling ependyma, the question arose whether this neoplasm originated from the ependyma or from the epithelium of the choroid plexus. Conclusive evidence of its origin from the latter is furnished by the transition of the choroid plexus into the neoplasm and by the collagenous connective tissue stroma of the growth. Moreover, the presence of a thin layer of collagenous connective tissue between the capsular epithelium and the brain tissue in all those places where the capsule was adherent to the ventricular wall proved conclusively that this epithelial lining was not identical with ependyma.

Mallory pointed out a valuable histologic point of differentiation between ependymoma and other types of glioma, namely, the presence of blepharoplasts in the former. Davis and Cushing,<sup>6</sup> Van Wagenen<sup>2</sup> and others used the same method to differentiate papillary ependymoma from papilloma of the choroid plexus. Blepharoplasts were absent in the case described here, although many epithelial cells carried cilia on their free surface. The latter fact does not speak against the origin of the tumor from the choroid plexus, since the epithelium of the choroid plexus is normally ciliated during fetal life, the time of origin of this neoplasm.

A comparison of all reported cases of papilloma of the choroid plexus in young infants shows a striking similarity of the essential features in almost all cases. Thirteen cases of this tumor, including the one reported here, have been described in which the patients were infants up to 2 years of age.<sup>7</sup> In 9 of these cases, the left lateral ventricle

6 Davis, L. E., and Cushing, H. *Arch Neurol & Psychiat* **13** 681, 1925

7 (a) Graves, G. W., and Fless, M. M. *Am J Dis Child* **47** 97, 1934  
 (b) Toepfich, G. *Frankfurt Ztschr f Path* **33** 238, 1925 (c) Bleyer, A., and Siebert, W. J. *J Pediat* **8** 193, 1936 (d) Stroeber, H. *Berl klin Wchnschr* **30** 123, 1893 (e) Goodhart, G. W. *Guy's Hosp Rep* **69** 217, 1918 (f) Weygandt. *Deutsche med Wchnschr* **43** 797, 1917 (g) Noodt, K. *Virchows Arch f path Anat* **258** 331, 1925 (h) Ebbs, J. H. *Arch Dis Childhood* **12** 403, 1937 (i) Van Wagenen<sup>2</sup> (j) Friedman and Solomon<sup>3</sup> (k) Turner and Simon<sup>4</sup> (l) Weinstein<sup>5</sup>

was the seat of the growth, in 3, the right lateral ventricle, and in 1, the fourth ventricle. Marked hydrocephalus was present in 11 cases. The reports of 5 cases mention marked atrophy of the cerebral cortex, with flattening of the gyri over the seat of the tumor. The average size of the growths was 3 to 5 cm. in diameter, but some had a diameter of 7 to 8 cm. The tumors were usually reddish brown, soft, friable and well outlined from the surrounding structures. In 5 cases, including the present case, the tumor was fixed to the wall of the ventricle. Four reports mention encapsulation of the neoplasm, while two others emphasize the absence of a capsule. Grossly visible hemorrhage from the tumor tissue was present in 3 cases, in 1 of which it was the immediate cause of death.

Microscopically, in all cases the neoplasm imitated more or less closely the structure of the choroid plexus. In all cases the predominant histologic picture was that of delicate branching papillae, each consisting of a core of vascularized connective tissue, lined by a single layer of columnar epithelial cells. The histologic picture in most cases was that of a benign, nonaggressive growth. In 2 cases, however, the neoplasm showed definite evidence of malignancy. The case published by Graves and Fliess<sup>7a</sup> was that of an anaplastic growth fixed to the brain tissue, Toeppich's case<sup>7b</sup> was that of a tumor completely filling out the fourth ventricle of a 2 year old child with definite atypism of the epithelial cells and numerous metastases throughout the brain and the spinal cord. In 2 cases the classification of the growth as benign or malignant is doubtful, since the neoplastic epithelium, while free from atypical changes, had invaded the brain tissue to a slight extent, these are the cases reported by Bleyer and Siebert<sup>7c</sup> and that reported in this paper. The former also showed a few "seed implants" in the vicinity of the tumor mass. Such implants, according to most authors, are not a sign of malignancy. Finally, the tumor described by Friedman and Solomon<sup>8</sup> is worth mentioning because of the presence of many multinucleated giant cells and the lack of uniformity of its epithelial cells. This neoplasm was not encapsulated and was attached to the floor of the descending horn by a broad base. Its surface was eroded and a massive hemorrhage had occurred from it.

Degeneration and swelling of some papillae were present in almost all cases. In Stroeber's<sup>7d</sup> case these degenerated papillae could be seen grossly as pea-sized gelatinous spheres embedded in tumor tissue.

#### SUMMARY

A case of papilloma of the choroid plexus in the left lateral cerebral ventricle of a newborn infant has been described, the tumor showed no histologic signs of malignancy but had invaded the brain tissue to a slight degree. The reported cases of papilloma of the choroid plexus in young infants are strikingly similar in their essential features.

# Laboratory Methods and Technical Notes

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## USE OF SOLID CARBON DIOXIDE IN PREPARATION OF GROSS PATHOLOGIC SPECIMENS

JOHN T BAUER, M D, PHILADELPHIA

With the commercial introduction of solid carbon dioxide ("dry ice") in 1925, methods were soon devised for using it in the preparation of frozen sections for microscopic examination,<sup>1</sup> but so far as I am aware no mention has been made of its application in the preparation of gross specimens. For those lacking suitable refrigeration who occasionally wish to prepare longitudinal or cross sections of large parts of cadavers, such as an extremity, in such a manner as to preserve the relationships and color of the bones and soft parts, the following methods can be recommended.

A fiber packing box or container large enough to hold the part to be frozen is obtained and lined with an old woolen blanket and a few newspapers. On this the specimen is placed, and then small pieces of solid carbon dioxide (5 to 25 cm in diameter) are placed about the specimen until it is entirely surrounded. (Heavy gloves should be worn while cracking the solid carbon dioxide and packing it about the specimen and while handling the specimen after it is frozen, to prevent severe frostbite.) The newspapers and blanket are then tightly wrapped about the solid carbon dioxide.

Depending on the temperature of the room and the size of the specimen, a block or two of solid carbon dioxide (50 to 100 pounds [22.7 to 45.4 Kg]) will suffice to freeze an arm or a leg solid within twenty-four to thirty-six hours. At the end of this time gross sections can be sawed in any plane through skin, subcutaneous fat, muscles, blood vessels and bone, without tearing or distorting of the tissue. (We have used a carpenter's crosscut saw with 8 or 9 teeth per inch.) The sawdust that adheres to the cut surface of the specimen is quite dry at first and can be scraped away with the edge of a knife.

If the cut surface is then gently and quickly washed with cold water, the water will be frozen into a thin smooth transparent layer of ice, which readily permits photography with few or no dazzling highlights or reflections. Color photographs of such specimens are especially pleasing, as the natural colors are preserved.

Blocks can then be taken from the frozen specimen with a saw and chisel and placed in the usual histologic fixatives for the preparation of material for microscopic study. No detrimental effect other than slight shrinkage has been noted in

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From the Ayer Clinical Laboratory of the Pennsylvania Hospital

1 Dunn, F L. J Lab & Clin Med **16** 627, 1931. Lindsay, J W, Rice, E C, and Selinger, M A. J A M A **96** 773, 1931.

the microscopic sections of tissue frozen by this means. Although chemical analyses of tissue frozen by solid carbon dioxide have not been made in this laboratory, they should be possible, as rapid freezing retards most chemical changes.

Modifications of the method outlined have been suggested but not tried. These include injection of the vascular bed of the specimen with fixatives before freezing and use of a band saw or other kinds of power saws instead of a hand saw.

The cost is little more than that of the solid carbon dioxide, which in our experience was between \$3 and \$3.50 per specimen.

The method described has been used in this laboratory on three occasions. On two, the specimen was a lower extremity removed at the hip joint because of an osteogenic sarcoma of the femur, and on the third occasion the specimen was an upper extremity removed at the



An upper extremity removed at the shoulder from a woman suffering with osteitis deformans (Paget's disease) associated with an osteogenic sarcoma of the lower end of the humerus.

shoulder from a woman suffering with osteitis deformans (Paget's disease) associated with an osteogenic sarcoma of the lower end of the humerus. A photograph of this specimen (figure) demonstrates the usefulness of the method by illustrating (1) the preservation of the relationship of the tumor in the humerus to the surrounding soft parts and (2) the almost complete absence of disturbing reflections usually seen in fresh tissue.

#### SUMMARY

A satisfactory method is outlined whereby gross sections of tissue can be prepared by sawing through the soft parts and bone after the entire specimen has been frozen with solid carbon dioxide ("dry ice").

## A SIMPLE METHOD OF PREPARING LANTERN SLIDES OF HISTOLOGIC SPECIMENS IN TWO COLORS

TRACY J. PUTNAM, M.D., BOSTON

The preparation of lantern slides in colors resistant to heat has become a simple matter since the introduction of kodachrome film in cut film sizes. A film  $2\frac{1}{4}$  by  $3\frac{1}{4}$  inches (5.7 by 8.2 cm) is large enough to fit into the mat of an ordinary slide, or two 35 mm films may be mounted on the same slide. There are still three objections to kodachrome, however. One is the matter of expense, which precludes its routine use in many laboratories. The second is that exposure must be accurate within narrow limits. Third, the success of the photograph can be judged only after the film has been returned from the processing plant.

An easy and inexpensive method of approximating the colors of microscopic slides stained with hematoxylin-eosin, methylene blue-eosin, polychromatic methylene blue or its substitutes, Mallory's connective tissue stain, Mann's stain and others in which the only tints represented are blue and light shades of red is to tone, mordant and stain an ordinary lantern slide plate. The method is borrowed from a method once used in the moving picture industry.<sup>1</sup> Blue or brown toning of slides is, of course, an old trick, and Jones<sup>2</sup> recently called attention to the usefulness of mordanted colors for lantern slides of histologic material.

The method is as follows:

A soft, well exposed negative is taken on fine grain panchromatic film with a Wratten E (orange) filter, at any desired enlargement. From the negative a soft positive lantern slide is prepared. It should be of such quality that practically no blacks are present. The blues should be represented by dark grays, reds and pinks, by extremely light tones. There should be some areas in the slide entirely free from deposits of silver, corresponding to empty spaces in the section. A weak ferricyanide bleach may be used to obtain these white spaces if necessary. The slide should be thoroughly fixed in fresh sodium thiosulfate (hyposulfite) and

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From the Neurological Unit, Boston City Hospital, and the Department of Neurology, Harvard Medical School.

This method was developed in the course of investigations financed by the Multiple Sclerosis Fund of Harvard University, including a grant from the John and Mary Markle Foundation.

<sup>1</sup> Tinting and Toning of Eastman Positive Motion Picture Film, ed. 4, Rochester, N. Y., Eastman Kodak Co., 1927.

<sup>2</sup> Jones, W. R. J. Lab. & Clin. Med. **23**: 1297, 1938.

hardener—best in two successive baths. It is then washed for one or two hours in running water. Incomplete fixation or the slightest trace of sodium thiosulfate is ruinous.

The slide is then toned in the following solution (Eastman formula T-18)

Ammonium persulfate	1 Gm
Ferric alum (ferric ammonium sulfate)	2.5 Gm
Oxalic acid	6 Gm
Potassium ferricyanide	2 Gm
Hydrochloric acid (10 per cent)	2 cc
Water to make	2,000 cc

The slide should be toned until the dark grays are blue, leaving light tones mordanted but unchanged in color. It is often well to leave a few points still black. It is washed ten to fifteen minutes (not longer) and then immersed in the following solution (Eastman formula T-17a)

Safranin <sup>3</sup>	0.2 Gm
Acetic acid (glacial)	0.5 cc
Water to make	1,000 cc

It is left in this solution until the light tones acquire the desired color (usually fifteen minutes). The blue may be intensified by washing and returning it to the iron bath, and the red may be made deeper by returning it to the safranin bath. Overstaining with safranin may be remedied by dipping in 0.2 per cent solution of concentrated ammonia, then washing, but there is no way of decreasing the intensity of the blue.

When the colors are satisfactory, the slide is washed for ten minutes and dried. A little yellow may be "dubbed in" if desired. The finished slide is given a coat of varnish and mounted in the usual fashion. The colors are permanent, and the slide is resistant to heat. The reds produced are somewhat brownish, but the lighter shades are satisfactory, and the effect is convincing.

For other combinations of colors which might be useful—for example, blue and yellow for Weigert-Pal slides, brown and yellow for silver stains—consult the manual on toning motion picture films.<sup>1</sup>

#### SUMMARY

A simple and inexpensive method of combined toning, mordanting and staining of ordinary lantern slide positives is described, which is applicable to histologic subjects stained by methods in which blues and reds alone are represented.

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3 Quantities under 1 pound (453 Gm) may be obtained from the Eastman Kodak Company, Rochester, N. Y. Larger amounts from the National Aniline Company, 40 Rector Street, New York.



## Notes and News

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**University News, Promotions, Resignations, Appointments, Deaths, etc**—Normand L. Hoerr, assistant professor of anatomy in the University of Chicago, has been appointed professor of anatomy in Western Reserve University, succeeding the late Thomas Wingate Todd.

Thomas B. Turner has been appointed professor of bacteriology in the school of hygiene and public health of Johns Hopkins University.

Raymond B. Allen, dean of Wayne University College of Medicine, Detroit, has been appointed executive dean of the Chicago colleges (medical, dental and pharmaceutical) of the University of Illinois.

Frederick P. Gay, professor of bacteriology in the College of Physicians and Surgeons of Columbia University, died on July 14, at the age of 65.

Stanley P. Reimann, Philadelphia, has been appointed chairman and pathologist of the newly established division of cancer control in the state department of health of Pennsylvania.

Alfred N. Richards, professor of pharmacology, has been appointed to succeed the late Alfred Stengel as vice president of the University of Pennsylvania in charge of medical affairs.

Elson B. Helwig and William O. Russell have been appointed instructors in pathology in Washington University School of Medicine.

**Awards**—The Theobald Smith medal and prize of \$1,000 has been awarded by the American Association for the Advancement of Science to Albert B. Sabin, associate of the Rockefeller Institute for Medical Research, in recognition of his work on pneumococcal infections.

The annual award of the American Pharmaceutical Manufacturers' Association was presented to Nathan B. Eddy and Lyndon F. Small, of the United States Public Health Service, at the recent annual meeting, in recognition of the "fundamental and outstanding studies of the chemistry, pharmacology and therapeutics of morphine derivatives for the alleviation of human suffering."

The French League against Cancer has awarded the Amerongen prize of 160,000 francs to A. H. Roffo, director of the Institute of Experimental Medicine for the Study and Treatment of Cancer at the University of Buenos Aires.

The Alvarenga Prize of the College of Physicians of Philadelphia has been awarded to Harry Goldblatt for his work on the genesis of hypertension.

**Beit Memorial Fellowships for Medical Research, London**—The first Beit fellow, now Sir Thomas Lewis, was elected in 1910. With the elections of 1938, the total of fellows reached 200, of these 30 were women. Of the 170 men, 6 have been elected to the Royal Society and 34 have been appointed university professors. The value of the fellowships varies from \$2,000 to \$2,500, approximately. The most frequent places of work by Beit fellows have been Cambridge University, London University and the Lister Institute of Preventive Medicine.

# Abstracts from Current Literature

TO SAVE SPACE THE ORIGINAL TITLES OF ABSTRACTED ARTICLES SOMETIMES  
ARE SHORTENED

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## Experimental Pathology and Pathologic Physiology

TRYPSIN INJECTION INTO RENAL ARTERIES M FRIEDMAN and L N KATZ,  
J Exper Med 68 485, 1938

Trypsin injected into both renal arteries of the dog was found to cause acute necrosis of large sections of the kidney, an immediate excretory insufficiency and transient hypertension. In dogs surviving the acute phase of the reaction to the injected trypsin a chronic renal excretory insufficiency developed, with no hypertension, despite the severity and duration of the renal excretory insufficiency. The application of a Goldblatt clamp to the renal artery of one of the two kidneys into which trypsin had been injected led to a rise in blood pressure, which returned at once to normal when the ischemic kidney was removed, even though the preexisting renal excretory insufficiency was augmented. This experience demonstrated unequivocally that chronic renal excretory insufficiency and hypertension are not directly related. The application of a Goldblatt clamp to the renal artery of one kidney and the simultaneous injection of trypsin into the other led to hypertension. The later removal of the ischemic kidney led to severe renal excretory insufficiency, at the same time the preexisting hypertension disappeared. This indicated again that renal excretory insufficiency and renal ischemia produce different phenomena and that the former had no direct relation to hypertension.

FROM AUTHORS' SUMMARY

A NEW METHOD OF PRODUCING RENAL INSUFFICIENCY AND HYPERTENSION IN  
THE RABBIT D R DRURY, J Exper Med 68 693, 1938

A simple method is described for producing renal insufficiency in rabbits by limiting the blood flow through one kidney and later removing the other. Renal insufficiency of any desired degree of severity can be obtained. The limitation of blood flow should be produced by allowing the artery to grow up to the size of a loop of thread laid around it when the rabbit is small. Attempts to reduce the blood flow by constricting the renal artery in the adult rabbit usually result in necrosis of the kidney. The induction of renal insufficiency is usually followed by loss of weight and disturbance of the apparatus for maintaining equilibrium. Hemorrhages into the intestinal wall and into the lumen of the intestine are frequent. A moderate degree of anemia, as judged from hematocrit readings, comes on very rapidly after reduction of the total kidney mass. The total red blood cell volume seems to assume a lower level, which is maintained for a long time. Hypertension is present before the ablation of the right kidney and becomes more severe after this operation. A certain degree of hypertension can be produced in the adult rabbit by moderate restriction of the renal blood flow of one kidney. The severity of this is increased by removal of the other kidney.

FROM AUTHOR'S SUMMARY

NUTRITIONAL CYTOPENIA IN THE MONKEY W C LANGSTON, W J DARBY,  
C F SHUKERS and P L DAY, J Exper Med 68 923, 1938

Young rhesus monkeys (*Macaca mulatta*) were given a diet containing casein, polished rice, whole wheat, salt mixture, sodium chloride, cod liver oil and ascorbic acid. They showed a syndrome of anemia, leukopenia and loss of weight. Ulcer-

ation of the gums and diarrhea were common, and death occurred between the twenty-sixth and the one hundredth day. Four monkeys were given the deficient diet supplemented with 1 mg of riboflavin daily, and these presented the characteristic signs and died in periods of time similar to the periods of survival of monkeys receiving the deficient diet alone. Nicotinic acid, either alone or in combination with riboflavin and thiamin chloride, failed to alter appreciably the course of the deficiency manifestations. Thus, it is evident that this nutritional cytopenia is not the result of a deficiency of vitamin B, riboflavin or nicotinic acid. The deficient diet supplemented with either 10 Gm of dried brewers' yeast or 2 Gm of liver extract (Cohn fraction G) daily supported good growth, permitted normal body development and maintained a normal blood picture over long periods. It is obvious that yeast and liver extract contain a substance essential to the nutrition of the monkey which is not identical with any of those factors of the vitamin B complex that have been chemically identified. The authors have proposed the term "vitamin M" for this factor which prevents nutritional cytopenia in the monkey.

FROM AUTHORS' SUMMARY

CORNEAL VASCULARIZATION IN RIBOFLAVIN LACK O. A. BESSEY and S. B. WOLBACH, *J. Exper. Med.* **69** 1, 1939

Vascularization of the cornea of the rat in the absence of antecedent pathologic damage is probably a specific, and the most reliable, criterion of riboflavin deficiency. Its initiation and repair may be used for testing the biologic activity of compounds structurally related to riboflavin. The fact that the invading capillaries are easily visible in the living animal and the fact that the growth and regression of the blood vessels are under dietary control and for a considerable period of time are unaccompanied by other pathologic reactions make this method very suitable for the study of problems related to capillary growth. Bessey and Wolbach believe that the best hypothesis in explanation is that the vascularization is a response to asphyxia of the tunica propria.

FROM AUTHORS' SUMMARY

A STUDY OF BONE REGENERATION GUSTAV LEVANDER, *Surg., Gynec. & Obst.* **67** 705, 1938

The specific osteoblastic theory holds that regeneration of bone may take place from preexisting cells belonging to the different layers, the metaplastic theory maintains that bone may be formed as a result of a process of transformation of nonspecific connective tissue. An essential part of the first theory concerns the predominant role of periosteum. Experimental evidence, however, has shown that it is only the periosteum of the growing skeleton that has the power to stimulate the formation of bone. Also it is known that when fully differentiated hard bone tissue is grafted, new bone is always formed. In a series of rabbits Levander first studied the mode of origin of new bone after transplanting into soft parts hard bone tissue stripped of periosteum. New bone formed from the mesenchymal tissue in the areas surrounding the graft. In his opinion, the specific stimulus necessary for the formation of new bone is brought to the mesenchyme in the form of a substance liberated from the graft and conveyed with the lymph into the surrounding areas. In a second series of experiments alcoholic extracts of bone tissue were injected into soft tissues of the abdominal wall as far as possible from any preexisting bone. Cartilage or bone was formed at the seat of injection in 22 per cent of the rabbits. Alcohol alone or alcoholic extracts of other tissues gave negative results in a control series. On the basis of these experiments it is felt that bone regeneration takes place as a result of activation of the nonspecific mesenchymal tissue by some specific bone-forming substance. This supports the heteroplastic theory of bone formation and conforms with the views of Spemann with regard to embryonic development.

WARREN C. HUNTER

## Pathologic Anatomy

CORONARY ARTERY DISEASE ANALYZED POST MORTEM W H GORDON, E F BLAND and P D WHITE, *Am Heart J* **17** 10, 1939

In a study of the records of 3,400 consecutive postmortem examinations made at the Massachusetts General Hospital between February 1925 and September 1937 the following facts were ascertained. After the age of 70 years there was no difference in the incidence of disease of the coronary arteries in men and women. Before that age there was much more of this disease in men than in women. The incidence and the degree of coronary atherosclerosis were found to be significantly greater in the 600 patients from the private departments than in the 2,800 patients from the general wards. The greatest difference occurred in the middle-aged series in which coronary occlusion was found to be twice as frequent in the private patients. This difference was most striking in middle-aged men. These observations are in general agreement with clinical impressions.

FROM AUTHORS' SUMMARY

PHARYNGEAL PITUITARY GLAND R H MELCHIONNA and R A MOORE, *Am J Path* **14** 763, 1938

A small mass of typical or atypical pituitary tissue was found in the pharyngeal mucosa in 51 of 54 unselected cases examined. The differentiated cells in the pharyngeal pituitary gland are histologically identical with those in the anterior lobe of the pituitary gland, but there are relatively few chromophilic cells. Under normal conditions of growth and activity it is unlikely that the pharyngeal pituitary gland has any significant physiologic function, but in some cases of altered structure or activity of the pituitary gland it cannot be denied that the pharyngeal pituitary gland may undergo structural alterations and serve as an endocrine organ.

FROM AUTHORS' SUMMARY

GIANT INTERSTITIAL CELLS OF THE TESTIS A A NELSON, *Am J Path* **14** 831, 1938

In a study of microscopic sections of 721 testes from a series of 470 autopsies on men 18 years of age or more, giant interstitial (Leydig) cells having from 4 to 30 (usually 8 or 10) nuclei were found in 85 testes. They were found at all ages and in all sorts of general disease conditions. They do not appear to have been described previously, although their existence has been hinted at. The observations of Berger and others on the "sympathicotropic" or "hilus" cells have been confirmed and extended. It is generally agreed that these cells in the testis are identical with the ordinary interstitial or Leydig cells. The author proposes the name "extraparenchymal interstitial cells" or "extraparenchymal Leydig cells" as best describing them.

FROM AUTHOR'S SUMMARY

PULSATING ANGIOMA (GENERALIZED TELANGIECTASIA) OF THE SKIN ASSOCIATED WITH HEPATIC DISEASE D H WILLIAMS and A M SNELL, *Arch Int Med* **62** 872, 1938

The association of cutaneous pulsating angioma (generalized telangiectasia) and hepatic disease is not uncommon. The association probably depends on two inherent developmental characteristics which are closely related. The cutaneous lesions are primarily angiomatous and have a border of radiating telangiectatic vessels. These lesions, the development of which represents a recessive characteristic, are identical with those of hereditary hemorrhagic telangiectasia, the development of which represents a dominant characteristic. The hepatic disease is usually chronic, with cirrhosis and atrophy of the hepatic parenchyma predominating. Alcohol, arsenic and syphilis are associated factors.

ARTERIOLES OF THE PANCREAS, LIVER, GASTROINTESTINAL TRACT AND SPLEEN IN  
HYPERTENSION C G MORLOCK, Arch Int Med 63 100, 1939

No measurable changes in the arterioles due to age were found in the pancreas, liver, gastroenteric tract or spleen in normal persons. Measurable thickening of the arterioles, with reduction of the ratio of the thickness of the wall to the diameter of the lumen, was found in hypertensive patients of all ages. In severer hypertension there was greater thickening of the walls, with greater reduction of the ratio. Histologically, in the early stages the change was hyperplasia of the media, and in the later stages, degeneration and fibrosis. In the more severely damaged vessels degeneration of the intima was prominent, the latter composing the greatest portion of the wall. Arterioles of equal caliber showed varying degrees of pathologic change. From a histologic standpoint the pancreas exhibited the most pronounced arteriolar changes, but as a tissue the pancreas suffered no more than the other organs. The clinical classification of hypertension on the basis of severity of symptoms is substantiated by the corresponding degree of pathologic change in the arterioles. No attempt is made to establish a causal relationship between hypertension and the arteriolar changes found with hypertension.

SABRO TASHIRO

THE MEDULLOBLAST AND THE MEDULLOBLASTOMA J KERSHMAN, Arch Neurol  
& Psychiat 40 937, 1938

From studies of embryos and fetuses by means of Hortega's silver method Kershman concluded that medulloblasts—the composite elements of medulloblastoma, considered hypothetic—actually exist. Medulloblasts occur only in the cerebellum, where they are identified with the cells of its external granular zone. These cells are bipotential, for they give rise to neuroblasts and spongioblasts. The latter are considered by Kershman medulloblasts which do not occur in any other part of the central nervous system—for instance, the spinal cord or the cerebral hemispheres—and for this reason give rise to medulloblastoma only in the cerebellum.

G B HASSIN

PERINEURIAL CYSTS OF THE SPINAL NERVE ROOTS I M TARLOV, Arch Neurol  
& Psychiat 40 1067, 1938

Tarlov described 5 cases of cysts of the sacral and coccygeal roots of the spinal cord. The age of the patients ranged from 49 to 63. The cysts varied in diameter from that of a pinhead to 2 cm and originated in the perineural spaces, which, according to Tarlov, are bordered by the arachnoid and pia. The walls of the cysts, which were continuous with the dural and arachnoid membranes, consisted of several layers of arachnoid cells with a delicate connective tissue framework, while the inner border was formed of flattened endothelial cells and endoneurial reticulum. Tarlov thinks that such cysts may account for the root pains of sciatica in some cases and that they probably have their origin in an inflammatory process which seals off a portion of the perineurial space.

G B HASSIN

GENESIS OF MICROGLIA IN THE HUMAN BRAIN J KERSHMAN, Arch Neurol &  
Psychiat 41 24, 1939

The microglia which is considered by some ectodermal, by others mesodermal, has been studied by Kershman with the silver method of Hortega in human embryos of from eight to twenty-nine weeks. He concluded that microglia is a mesodermal element and originates from certain areas located underneath the meninges, in connection with inferior choroid plexus around blood vessels, in short, wherever the cerebral and mesodermal tissues are in near proximity. The various stages of growth of a typical microglial cell were traced. Where youngest it appears in an ameboid form, which is gradually transformed into a typical microglia cell. The earliest appearance of microglia seems to occur simultaneously

with that of the intracerebral blood vessels. The precursor of the microglial elements are wandering mesodermal elements, the histiocytes of mesodermal tissues. In short, microglia cells are derived from embryonic mesenchymal cells.

G B HASSIN

THE HEMATOENCEPHALIC BARRIER L S KING, Arch Neurol & Psychiat **41** 51, 1939

Trypan blue injected intravenously into animals colors the dura, the stroma of the choroid plexus and the parenchyma of every organ in the body except the brain. Here only the anterior half of the pituitary body, the pineal body and the area postrema become blue. On the other hand, dye injected into the subarachnoid space causes marked coloring of the cerebral parenchyma. King attempted to explain only the effects of intravenous injections. He holds that trypan blue is attracted only by organs that are relatively rich in connective tissue (the dura or the choroid plexus, for instance). In other words, the affinity of the connective tissue for trypan blue is the cause of this phenomenon and not the so-called hemato-encephalic barrier which is supposed to be put up by the endothelium and to prevent the dye from passing into the parenchyma. There is increased affinity for trypan blue in inflammatory conditions of the brain. With long-continued administration of trypan blue many cells within the parenchyma stain vitally, especially around the central canal and cornu ammonis, where the dye is flocculated by "suitable" cells.

G B HASSIN

EARLY PHASES OF PROSTATIC HYPERPLASIA C L DEMING and C NEUMANN, Surg, Gynec & Obst **68** 155, 1939

The material for the study of the early lesions of prostatic hyperplasia was obtained at autopsies on subjects of 45 years and upward. Whole sections of glands were stained with hematoxylin-eosin and Masson's trichrome stain, the latter to differentiate muscle from fibrous tissue. Glands containing minute, early lesions were sectioned serially. The first change in benign enlargement of the prostate is primarily a multiplication of fibromuscular elements, resembling the early stages of uterine fibromyoma. The posterior part of the urethra and the internal vesical sphincter contain muscle fibers which are derived from the same embryologic source as those of the uterus. The glandular portion of the tumor is derived from ducts adjacent to the fibromyomatous nodule and not from glands. The suburethral glands of Albarran are not involved in the early stages. It is probable that the solid nodule produces some stimulating effect on the epithelium of the duct wall, causing its epithelium to bud and invade the solid nodule and form glands within it. The fibromyomatous tissue is overgrown by a more rapidly growing duct and glandular tissue, with the result that the nodule in its later stages acquires the appearance of an encapsulated glandular tumor.

WARREN C HUNTER

RUPTURE OF ANEURYSM INTO PITUITARY ADAMANTINOMA H BUSS, Beitr z path Anat u z allg Path **101** 335, 1938

Buess reports a unique case of fatal rupture of an aneurysm of the right anterior cerebral artery into an intrasellar cystic pituitary adamantinoma. The clinical and pathologic manifestations are discussed.

R J LEBOWICH

PANCREAS, HYPOPHYSIS AND THYROID IN CHILDREN OF DIABETIC MOTHERS H OKKELS and E BRANDSTRUP, Acta path et microbiol Scandinav **15** 268, 1938

The pancreatic islets in children of diabetic mothers are larger and more numerous than is normal. Accompanying the insular hypertrophy there is an

increase in the differentiated epithelium of the anterior lobe of the pituitary. The thyroid gland also shows signs of increased activity. All these alterations have a common cause, probably the hyperglycemic condition induced in the fetus. This condition possibly effects the changes observed in the endocrine glands by the intermedium of the central nervous system.

FROM AUTHORS' SUMMARY

### Microbiology and Parasitology

MURINE AND HUMAN LEPROSY. A. W. SELLARDS and H. PINKERTON, *Am J Path* 14: 421 and 435, 1938

Inoculation of murine leprosy into the brains of monkeys (*Macacus rhesus*), rabbits, white rats, ordinary white mice and guinea pigs resulted in the development of progressive lesions in all except the guinea pigs. White mice of mixed breeds were also readily infected by inoculation in the spleen, the liver or the peritoneal cavity, but on subcutaneous inoculation they usually showed only an abortive infection. Mice of some inbred strains frequently acquired well marked local lesions on subcutaneous inoculation, and subsequently showed extensive metastases. Murine leprosy has been maintained by serial passage in white mice of mixed breeds for four years. Various routes of inoculation have been used. Massive infections have developed, providing excellent material for experimental purposes. The bacillus of rat leprosy produced infection in the murine rodents, white rats and white mice, according to the manner of a micro-organism developing in a favorable host, but in rabbits and monkeys the circumstances of its multiplication were apparently less favorable. Monkeys (*M. rhesus*) inoculated with human lepromatous material were kept under observation for more than one year. *Lepra* bacilli were seen in small numbers in smears of inguinal glands. Transfers of the infection were made to other monkeys, in which similar low grade infections developed during a period of more than a year. Portions of the pia mater of one of these monkeys were implanted intracerebrally in white rats, fifteen months later many acid-fast bacilli were found in the brain of 1 rat. Briefly, human *lepra* bacilli were maintained in animals for a period of three and three-fourths years, but no progressive disease and no active lesions developed.

The *lepra* cells in murine leprosy are derived largely from mesenchymal cells belonging to the reticuloendothelial system. Exceptionally, however, epithelial cells, specifically those of the epidermis, testicular tubules and epididymis, became distended with *lepra* bacilli. This observation suggests that the relative resistance of epithelial cells to infection may depend on the inability of *lepra* bacilli to enter them rather than on intracellular conditions unfavorable to their growth. In infected rats and mice surviving for a long time the tissues of practically all the organs were extensively replaced by nonvacuolated *lepra* cells distended with bacilli, but renal tissue contained relatively few of these cells. In the progressive local and metastatic lesions produced with *Bacillus leprae murium* in rabbits and monkeys the *lepra* cells were often vacuolated, and acid-fast bacilli were much less numerous in the lesions in these animals than in those produced in rats and mice, and were found only after prolonged search. Nonpathogenic acid-fast bacilli injected intracerebrally were taken up by macrophages and neutrophils but disappeared from the lesions in a few weeks, never producing metastatic lesions. Virulent tubercle bacilli, although innocuous in mice when injected subcutaneously, produced progressive and metastatic infection when injected intracerebrally. The lesions were noncaseating, and the tubercle bacilli were found largely within macrophages, so that these lesions closely resembled those of leprosy.

FROM AUTHORS' SUMMARIES

RELATION OF HUMAN ENCEPHALITIS TO ENCEPHALOMYELITIS IN HORSES C M EKLUND and A BLUMSTEIN, J A M A **111** 1734, 1938

In the summer of 1937 encephalitis occurred in 6 farmers in localities where equine encephalomyelitis was prevalent. The blood serum of 1 of 3 patients whose serums were tested was shown to neutralize the western strain of equine encephalomyelitis. As far as is known, this is the first time that neutralization of the virus of equine encephalomyelitis by human serum has been found.

FROM AUTHORS' SUMMARY

HUMAN ENCEPHALITIS EIGHT FATAL CASES, WITH FOUR IN WHICH THE DISEASE WAS DUE TO THE VIRUS OF EQUINE ENCEPHALOMYELITIS C WESSELHOEFT, E C SMITH and C F BRANCH, J A M A **111** 1735, 1938

Fatal acute encephalitis was observed in 8 patients. All the patients resided in an area where an epidemic of the eastern strain of equine encephalomyelitis involved more than 200 horses during the same period and where mosquitoes were unusually prevalent. Autopsies were performed on 7 of these 8 patients, and the virus of the eastern strain of equine encephalomyelitis was recovered from the brains of 4. The virus of the eastern strain of equine encephalomyelitis causes in human beings a profound acute disseminate and focal encephalomyelitis, characterized by intense vascular engorgement, perivascular and parenchymatous cellular infiltration and extreme degenerative changes in the nerve cells. The gross pathologic manifestations are not specific. When the infection is severe, the microscopic reaction can readily be distinguished from any of the common types of encephalitis.

FROM AUTHORS' SUMMARY

PYOGENIC FILTERABLE AGENT IN THE ALBINO RAT W H WOGLOM and J WARREN, J Exper Med **68** 513, 1938

A filtrable agent resembling virus is described. It was encountered in sarcoma 39, a propagable neoplasm of the white rat, and has now been maintained in this species for twenty-eight passages over a period of some seven months without appreciable loss in virulence. Its chief effect is the production of large abscesses in an animal species comparatively resistant both to viral diseases and to suppuration. The white mouse is more susceptible than the white rat, the rabbit less so, and the guinea pig is highly resistant. The agent was repeatedly recovered from sarcoma 39 when this was treated in special ways, but under the ordinary circumstances of routine transplantation it did not manifest itself. As yet there is no certainty as to where it came from or how it maintains itself under natural conditions.

FROM AUTHORS' SUMMARY

ULTRACENTRIFUGATION OF VACCINE VIRUS E G PICKELS and J E SMADEL, J Exper Med **68** 583, 1938

Ultracentrifugal studies on the CL dermal strain of vaccine virus indicate the following characteristics of the elementary bodies. A stable suspension of Paschen bodies in a dilute buffer solution of  $p_H$  6.2 to 8 sediments with the formation of a characteristic primary boundary which consistently shows a spread of approximately 14 per cent. The principal sedimentation boundary is accompanied frequently by one or several more rapidly moving boundaries which probably are produced by groups of agglutinated elementary bodies consisting of two or more particles. Occasionally the principal boundary may exhibit an irregular or peculiar behavior, a fact which necessitates a careful selection of material and the performance of many experiments for accurate interpretation of results. The sedimentation constant of the slowest moving particles forming the principal boundary is computed to be  $49.1 \times 10^{-11}$  cm/seconds/dyne. On the basis of this sedimentation rate the average diameter of the smallest virus particles in appre-



cialable amounts is estimated at 236 microns. If the boundary spread is due principally to slight differences in particle size, then the largest single elementary bodies are approximately 252 microns in diameter.

FROM AUTHORS' SUMMARY

CORRELATION BETWEEN THE GROUP SPECIFIC SUBSTANCES AND BIOCHEMICAL CHARACTERISTICS OF HEMOLYTIC STREPTOCOCCI M C HITCHLER and M A FARRELL, *J Infect Dis* **63** 225, 1938

"A study of the serological and biochemical properties of 42 strains of hemolytic streptococci led to the following conclusions:

"By means of the precipitin test 37 of the 42 strains are classified into the 7 serological groups of Lancefield.

"Five of the 7 serological groups, in a large majority of cases, show a definite relation to the source of the strains.

"The biochemical and cultural characteristics of all strains within a group are somewhat variable although predominant group reactions are noted. Certain correlations are indicated between the group specific substances and fermentative reactions."

AUTHORS' SUMMARY

TRICHINOSIS IN CLEVELAND. POSTMORTEM EXAMINATION OF DIAPHRAGMS AND SKELETAL MUSCLES FROM 100 CONSECUTIVE AUTOPSIES C H EVANS JR, *J Infect Dis* **63** 337, 1938

"An incidence of 36% of trichinosis for the Cleveland area is indicated by postmortem examination of diaphragms and skeletal muscles from 100 consecutive autopsies.

"In this series the usual procedure of examination of diaphragm by the digestion-Baermann method alone revealed an incidence of 20%.

"The application of the compression-microscopic and the digestion-Baermann methods shows variations in the number of organisms recovered, but together they give a more nearly true result than with either alone.

"The examination of skeletal muscles such as the intercostals and the sternomastoid in addition to the diaphragm gives a higher incidence of infestation than is revealed by examination of the diaphragm alone."

AUTHOR'S SUMMARY

VIABILITY OF PNEUMOCOCCI IN DRIED SPUTUM E G STILLMAN, *J Infect Dis* **63** 340, 1938

Pneumococci have been recovered from dried sputum which has been exposed to diffuse daylight at room temperature for months.

Pneumococci will live in dried sputum stored in the cold, on an average, four or five times longer.

FROM AUTHOR'S SUMMARY

EPIDEMIC INFLUENZA VIRUS W SMITH and C H ANDREWES, *Brit J Exper Path* **19** 293, 1938

An antigenic analysis of 28 strains of influenza virus isolated from human patients has been made by means of cross neutralization tests in mice. Many antigenic components enter into the constitution of the various strains. Of these components, four appear to possess major significance, they were found in very different proportions in the different strains. On the basis of their content of the four major antigens it was possible to classify the strains into three main categories, namely, highly specific strains, relatively nonspecific strains and intermediate strains. A strain of virus can be shown to absorb from a heterologous serum only those antibodies which correspond to its own antigenic components. Active immunization experiments with mice show that the protection conferred by vaccination with a single strain is most effective against that strain. The

efficacy of a vaccine against a heterologous virus is largely dependent on the closeness of antigenic relation between the strains concerned. Experiments with ferrets suggest that in animals with some basic immunity the response to vaccination may be less specific than that in animals with no previous experience of influenza. The difficulties of applying the results of animal experiments to human vaccination are discussed.

FROM AUTHORS' SUMMARY

MICROSCOPIC OBSERVATIONS ON THE BACTERIOPHAGE OF BACTERIUM COLI K B MERLING-EISENBERG, Brit J Exper Path **19** 338, 1938

The lytic action of the bacteriophage of *Bacterium coli* has been observed and photographed in the following stages. Particles appear within the bacterial cell, which swells up and finally bursts, discharging the particles into the surrounding medium. The bacteriophage is distinguished from nonspecific particles, and its size is found to be 25 millimicrons.

FROM AUTHOR'S SUMMARY

THE VIRUS OF TRACHOMA A CUENOD and ROGER NATAF, Arch Inst Pasteur de Tunis **27** 284, 1938

These studies confirm the authors' preceding studies of the presence of rickettsia-like bodies in trachoma. Cultures in series on the chorioallantoic membrane of the egg show lesions on the membrane, virulence for the embryo and frequent presence of rickettsioid bodies. This cultivation permits new analogies to be found between the etiology of trachoma and that of classic and murine typhus and permits the formulation of the hypothesis that epithelial inclusions of the Prowazek and Halberstaedter types and rickettsias and rickettsioid bodies are different stages in the development of the same virus. The studies suggest also the use of potassium cyanide in the treatment and prophylaxis of trachoma and of various infections due to rickettsias.

FROM AUTHORS' SUMMARY

### Immunology

FATAL ANAPHYLACTIC SHOCK IN MAN \*J ZISKIND and H J SCHATTENBERG, Arch Int Med **62** 813, 1938

Fatal anaphylactic shock followed a second intravenous injection of a foreign protein. At necropsy marked congestion of the liver and more moderate congestion of the other organs were noted. General enlargement of the lymph nodes and persistence of the thymus gland were also noted. The significant microscopic changes were as follows. The hepatic sinusoids were dilated and congested, as were also the alveolar capillaries of the lungs. Within these vessels the leukocytes and the eosinophils were apparently relatively increased. The alveoli in some areas were compressed, those in others, dilated. In some instances their walls were ruptured. In the spleen the malpighian corpuscles were increased in size and had large germinal centers. It is suggested that the mechanism of death was of the so-called dog type, namely, capillary dilation, especially in the liver, with corresponding fall in blood pressure and cardiac failure.

FROM AUTHORS' SUMMARY

PREVENTION OF HYPERSENSITIVITY IN TUBERCULOSIS R H FOLLIS JR, Bull Johns Hopkins Hosp **63** 283, 1938

Animals infected with the tubercle bacillus and prevented from becoming hypersensitive by treatment with old tuberculin show less caseation in their lesions than do control animals in which hypersensitivity has developed. In some of the nonhypersensitive animals pulmonary lesions develop which are marked by extraordinary proliferation of the acid-fast bacilli. Since these lesions did not develop

in the majority of the nonhypersensitive animals and since the same lesions were found in some of the animals of the control groups, shown to be hypersensitive, it is concluded that lack of hypersensitivity does not play a role in producing lesions of this type

FROM AUTHOR'S SUMMARY

POLYSACCHARIDES FROM TUBERCLE BACILLI F R SABIN, A L JOYNER and K C SMITHBURN, *J Exper Med* **68** 563, 1938

Purified tuberculopolysaccharides are relatively innocuous both to normal and to tuberculous guinea pigs. Both tuberculopolysaccharide and polysaccharides from pneumococci call larger numbers of leukocytes from the blood vessels than do salt solution and dextrose and trehalose. The mechanisms controlling the delivery of lymphocytes and neutrophils into the blood stream are different. Slight irritation of the peritoneal lining slows the delivery of lymphocytes to the blood stream. There are two phases in the reaction of the bone marrow to intraperitoneal injections. Correlated with the draining of neutrophils from vessels to tissues, owing to the presence of foreign materials in the latter, there is a draining of young neutrophils from the marrow into the sinuses of the marrow as these same materials reach the sinuses. The subsequent disintegration of the neutrophils extravasated into the tissues is correlated with increased myeloid activity in the marrow.

FROM AUTHORS' SUMMARY

REACTIONS TO TUBERCULOPROTEINS AND TUBERCULOPHOSPHATIDE K C SMITHBURN and F R SABIN, *J Exper Med* **68** 641, 1938

Prior observations on the cellular reactions to tuberculophosphatide are confirmed and the reactions compared with reactions induced by this material in tuberculous animals. In the latter the response is accelerated and augmented and simulates the Koch phenomenon. Tuberculoprotein produces no macroscopic reaction in normal animals. The microscopic reaction of neutrophils and monocytes regresses in less than a week. The same material in tuberculous animals causes a response characterized by more or less hemorrhage and necrosis, tissue degeneration and infiltration by neutrophils and monocytes. Late in the reaction there may be a few epithelioid cells and foreign body giant cells. Preparations of tuberculophosphatide which contain no tubercle bacilli or only a few induce the typical cellular response but do not induce hypersensitiveness to tuberculin. Repeated intradermal skin test injections of tuberculoprotein MA-100 in normal guinea pigs may be followed by mild hypersensitiveness to subsequent injections.

FROM AUTHORS' SUMMARY

RESPONSE OF IMMUNIZED MICE TO REINOCULATION WITH THE VIRUS OF ST LOUIS ENCEPHALITIS E A COOK, *J Infect Dis* **63** 206, 1938

If seven to twenty-one days after mice have been given one or two injections of the virus of St Louis encephalitis by either the subcutaneous or the intraperitoneal route they are reinoculated intracerebrally or intranasally, they may show sudden flaccid paralysis of the limbs, with a fatal or a nonfatal outcome. Similar results may be observed on intracerebral inoculation of mice after one or two previous intranasal inoculations. The incubation period of the paralytic condition is five to fourteen days, being shorter (five to eleven days) after intracerebral than after intranasal injection (eleven to fourteen days).

Suspensions of the brains or cords of such paralyzed animals when injected into normal mice give rise to typical encephalitis. Virus exists usually in slightly higher relative titer in the cord than in the brain but is in comparatively low titer in both and disappears rapidly. It is not detectable in the central nervous system seven, fourteen or twenty-one days after the injection of a nonlethal dose.

The localization of the paralytic symptoms is not correlated with the site of injection.

The serum of mice given a single subcutaneous or intranasal injection of a nonlethal amount of virus possesses little or no neutralizing capacity. However, in animals so treated a certain degree of immunity exists, as shown by their resistance to subsequent inoculation with virus in amounts rapidly lethal to controls.

The chief pathologic changes in the cords of paralyzed mice are vascular congestion, perivascular and diffuse round cell infiltration, proliferation of microglia and nerve cell degeneration, especially in the anterior horns.

FROM AUTHOR'S SUMMARY

LETHAL TOXINS OF HEMOLYTIC STREPTOCOCCI E W TODD, *Bull J Exper Path*  
**19** 367, 1938

Group A hemolytic streptococci produce two distinct varieties of streptolysin, which are both lethal to mice: (1) streptolysin O, which is sensitive to oxygen, (2) streptolysin S, which is extractable with serum. These two streptolysins are neutralized by separate, entirely unrelated antibodies. Antistreptolysin O protects mice against either death or hemoglobinuria which may be caused by streptolysin O, but it does not protect against streptolysin S. Antistreptolysin S protects mice against either death or hemoglobinuria which may be caused by streptolysin S, but it does not protect against streptolysin O. Antistreptolysin S does not protect mice against living cultures of hemolytic streptococci, and it does not enhance the protective action of antistreptolysin O.

FROM AUTHOR'S SUMMARY

ANTIRABIC IMMUNIZATION I J KLIGLER and H BERNKOPF, *Bull J Exper Path*  
**19** 378, 1938

Experiments are reported which indicate that formaldehydized tissue cultures of rabies virus possess considerable immunizing capacity for mice if injected intraperitoneally. The immunity to an intracerebral infection is incomplete, a little less than half of the mice given five to seven injections of the vaccine possessed solid immunity to 10 lethal intracerebral mouse doses, about 42 per cent possessed no immunity. Immunity to an intraperitoneal or subcutaneous infection is more easily established. Even a single intraperitoneal treatment with the inactivated culture virus is sufficient to protect mice against an intraperitoneal infection with a dose of fresh virus which kills about two thirds of the control mice. A similar immunity is conferred against a subcutaneous infection except that a larger amount of vaccine is required. Of special interest, perhaps, are the data showing that immunized mice which survive an intraperitoneal infection possess a greater degree of immunity than do untreated mice which survive such an infection. Primary treatment with inactive virus affects to a marked extent the immunity of these mice to an intracerebral infection. Mice which received diluted vaccine (1:10) or fewer doses possessed a lower degree of immunity than the others, although all of them survived an intraperitoneal or subcutaneous infection with fresh brain virus. Only half of the mice which survived an intraperitoneal infection without previous vaccination were immune to an intracerebral infection, whereas 84 per cent of previously immunized mice proved immune. A single dose of a rabies virus culture which had a very low virulence for mice, if injected intraperitoneally, conferred on the treated mice a high degree of immunity against a subsequent intracerebral infection with test brain virus.

FROM AUTHORS' SUMMARY

## Tumors

LUNG TUMOR DEVELOPMENT IN A RESISTANT STRAIN OF MICE M G SEELIG and  
E L BENIGNUS, *Am J Cancer* **34** 391, 1938

A 10 per cent mixture of carcinogenic tar in lampblack failed to induce cancer of the lung in any of 100 genetically tumor-resistant mice which inhaled the mixture over a period of ten months and which at autopsy showed a heavy deposition

of the lampblack in the lungs. This result is in marked contrast to that of a previous experiment in which with chimney soot there was induced an 8 per cent incident of pulmonary tumors in a less resistant strain of mice. A 4 per cent spontaneous incidence was observed in a control group of 50 animals in spite of the fact that this strain when living under a Bar Harbor environment showed less than 1 per cent incidence of tumor of the lung. It is entirely possible that the difference of tumor incidence in this and in our earlier experiment is due to (a) the varying tumor productivity of the particles used in the first experiment as contrasted with the softer, less traumatizing particles of lampblack used in the second experiment. Because pulmonary growths in mice may be so small as to escape macroscopic detection, it is believed that serial sectioning and microscopic examination of tissue are essential in determining the incidence of tumor of the lung. Unfortunately, it seems to be true that environmental conditions alter the genetic reaction to tumor growth on the part of even pure strain animals. Equally unfortunate is the lack of agreement of opinion, or at least of certainty, regarding the histology of pulmonary tumors in mice.

FROM AUTHORS' SUMMARY

TRANSPLANTABLE LYMPHOSARCOMA IN MICE. M. R. LEWIS, *Am J Cancer* **34** 399, 1938

Lymphosarcoma developed in 3 mice that were given injections of 0.8 mg of 1,2,5,6-dibenzanthracene dissolved in 0.4 cc of sterile olive oil. Tumors (Carnegie 172, 226 and 233) arose at the sites of injection seventy-five, one hundred and fifteen and two hundred and thirty-seven days later, respectively. They were composed of malignant lymphocytes. Two of them arose from the axillary lymph nodes and the third from the thymus. One of them (no 172) was transplanted through twelve generations in mice of the strain in which it originated. It was found to be 100 per cent transplantable in mice of the same strain but not transplantable in mice of other strains. The tumor was passed by implantation of whole blood or pieces of lung, liver, spleen, kidney, lymph node or tumor but not by implantation of blood plasma or of the supernatant fluid of centrifuged extracts of the tumor.

FROM AUTHOR'S SUMMARY

FREEDING WALKER TUMOR FROM CONTAMINATING BACTERIA. R. E. GARDNER and R. R. HYDE, *Am J Cancer* **34** 442, 1938

Metastases are readily obtained by inoculating small pieces of Walker tumor no. 256 into the tails of young susceptible rats. When the implanted tumor is contaminated with bacteria the metastases usually prove to be sterile, especially those from the region of the retroperitoneal lymph node.

FROM AUTHORS' SUMMARY

MALIGNANT GIANT CELL TUMOR OF BONE. F. W. STEWART, B. L. COLEY and J. H. FARROW, *Am J Path* **14** 515, 1938

The authors have been unable to arrive at a satisfactory descriptive term for the malignant giant cell tumors of bone. They believe that it is perhaps best to retain the designation "malignant giant cell tumor," since it carries at least a definite connotation. They state that the efforts to establish hard and fast lines of distinction for cells involved in bone development have made the description of the histogenesis of bone most complex. Through the ultracytologic analyses of various histologists, according to the authors, cells have acquired individualities which they probably do not merit or merit only in a transient sense. The cell elements of giant cell tumors cannot be separated from the connective tissue cells and vessels which are involved in the histogenesis of bone and which evolve in different directions, dependent on the physicochemical conditions of the period. The authors are much in sympathy with the views of Moschowitz as expressed in his paper on the relation of angiogenesis to ossifica-

tion, and they see many similarities in the development of malignant giant cell tumors. They doubt that one can specifically state that a giant cell tumor is a tumor of giant cells, intervening connective tissue cells or angioblastic elements or that the malignant giant cell tumor is a sarcoma of giant cells or of angioblastic elements or an endothelioma or a granulation tissue sarcoma, since there is great difficulty in separating the elements of the tumor into permanent entities. In their own cases they observed no true bone formation, and yet it would not surprise them if a tumor with this evolutionary pattern should appear. Despite the tendency, which they also have followed, to reject as giant cell tumors of malignant type those tumors in the metastases of which cartilage has appeared, they think the rejection may not be necessarily warranted.

Thus the form assumed by the process known as giant cell tumor will be found to depend on the nature of the circumstances, physical and chemical, which have initiated the process plus the extrinsic factors that have interfered with its normal evolution. Until the giant cell tumor and its malignant evolution are better understood, the interpretation of this type of tumor must remain in a speculative phase.

FROM AUTHORS' SUMMARY

CARCINOMA IN THE LEOPARD FROG B. LUCKL, J. Exper. Med. **68** 457, 1938

An epithelial tumor with acidophilic intranuclear inclusions frequently occurs in the kidneys of leopard frogs. This tumor usually has the appearance of an infiltrating and destructive adenocarcinoma. When large, it not uncommonly metastasizes, less often it is more orderly and adenomatous. When inoculated as living fragments or as a cell suspension into the lymph sacs, cranial cavity or abdomen, it gives no significant local growths, and the implanted material is resorbed. However, in approximately 20 per cent of the frogs surviving inoculation for more than six months, renal tumors develop which are like the "spontaneous" neoplasms. The incidence far exceeds that in the controls. Desiccated and glycerinated tumor injected into the abdomen gives the same result as living tumor. In somewhat over 20 per cent of animals surviving more than six months renal tumors occur. In alien species of frogs, no such tumors are produced by inoculation of either living or desiccated tumor. These experiments indicate the probability that the tumor of the kidney of the leopard frog is caused by an inclusion-forming, organ-specific virus.

FROM AUTHOR'S SUMMARY

PRIMARY RETICULUM CELL SARCOMA OF BONE F. PARKER JR. and H. JACKSON JR., Surg., Gynec. & Obst. **68** 45, 1939

The type of primary tumor of bone described apparently constitutes a definite group, hitherto unrecognized and unprovided for in the classification of the Bone Sarcoma Registry. Although such a tumor was suggested as a possibility in 1931, the first positive diagnosis of it was not made until 1936. Prior to this tumors of this type had been variously classified as Ewing's sarcoma, Hodgkin's disease, lymphosarcoma, osteogenic sarcoma, leukosarcoma or inflammation. Because of the more serious lesions with which it may be compared, the characteristics and clinical course in 17 cases, in 10 of which it involved males, are described. Primary reticulum cell sarcoma of bone differs materially from the generalized form in that in the great bulk of cases it occurs in persons under the age of 40 and involves the long or flat bones instead of the vertebrae and skull. Despite local extensive involvement and roentgenographic evidences of a massive and destructive lesion, the patient remains in good condition.

Histologically, the neoplastic cells have round, oval, indented or lobulated nuclei, twice the size of a lymphocyte, scattered chromatin granules and a considerable amount of cytoplasm. Despite the apparently malignant nature of the tumor it is quite amenable to treatment, as proved by the fact that 13 of the 17 patients are alive from six months to fourteen years after the first appearance of symptoms. Early diagnosis by biopsy followed by immediate amputation and irradiation seems to be the proper procedure.

WARREN C. HUNTER

BONE SARCOMA FACTORS INFLUENCING THE PROGNOSIS C C SIMMONS, Surg,  
Gynec & Obst 68 67, 1939

This report is based on the results of treatment of 47 primary malignant tumors of the long bones, excluding plasma cell myeloma, at the Massachusetts General Hospital during the period between 1920 and 1932. The prognosis is not as poor as is generally believed if the tumors are removed by radical surgical operation, it depends more on the amount of differentiation of the cells comprising the major portion of the tumor than on any one other factor. Tumors composed largely of adult fibrous tissue or cartilage offer a better prognosis than those in which the cells show marked anaplasia. Of 28 patients with osteogenic sarcoma on whom amputation was done, 39 per cent are living and without evidence of disease five or more years after operation. All patients (5) in whose tumors fibrous tissue predominated and on whom amputation was done are well, the percentage for those whose tumors showed a predominance of cartilage and who were treated in the same manner is 70, only 5.5 per cent of those with tumors made up of anaplastic cells are alive after five years. All patients with Ewing's sarcoma, whether treated by amputation or by irradiation, died of the disease.

WARREN C HUNTER

INFLAMMATION AND CARCINOGENESIS S BECK, Brit J Exper Path 19 319, 1938

Mice were given injections of 3,4-benzpyrene dissolved in a 30 per cent solution of turpentine in olive oil. In each instance a sterile abscess was thus induced around the benzpyrene. In other groups benzpyrene was injected into or near such abscesses. Two groups given injections of benzpyrene in the same solvents but without turpentine served as controls. In another control group abscesses were induced but no benzpyrene was injected. All groups receiving injections of benzpyrene acquired tumors and showed the fluorescence of persistent benzpyrene in the tissues for about six months. The following conclusions are drawn. Acute inflammation had no apparent influence on the rate of absorption or on the carcinogenicity of 3,4-benzpyrene in fatty solvents, the inflammatory fibrous tissue did not accelerate or retard the development of sarcoma, sarcoma did not originate from the fibrous tissue of subacute inflammation, 3,4-benzpyrene did not act at the locus of the injection but on tissues in the immediate neighborhood, through which it passed on its way to absorption. It is concluded that 3,4-benzpyrene titrates itself against the tissues, and an optimal concentration for an optimal time determines which of the susceptible cells become malignant. Probably only a few points in the tissue fulfil these conditions.

FROM AUTHOR'S SUMMARY

THE ANTIGENIC BASIS OF TUMOR TRANSPLANTATION P A GORER, J Path  
& Bact 47 231, 1938

Gorer studied the behavior of a malignant tumor and leukemic tissue arising in one strain of mice when this tumor and leukemic tissue were injected into mice belonging to an unrelated pure line and also when they were injected into other members of the strain in which the tumor arose. As a rule, members of the same strain of mice were susceptible, whereas unrelated mice were resistant. In resistant mice the injection of leukemic tissue and blood gave rise to immune isoagglutinins capable of acting on the red blood cells of mice belonging to susceptible species, the isoagglutinins attaining their maximum titer seven days after the injection. Following inoculation of sarcomatous tissue the maximum titer of the isoagglutinins corresponded with the time of complete regression of the tumor, i. e., fourteen days. The highest titers were obtained after tumor grafting, and leukemic tissue gave rise to stronger isoagglutinins than blood. The titers dropped rapidly during the week following the attainment of their maxima. The serologic evidence obtained in the study agreed with the genetic evidence published in a previous paper indicating that two genes govern the transplantability of the tumor and also with the hypothesis that both genes determine isoantigenic differences.

A S WIENER

HISTOLOGIC TYPES OF MENINGIOMA J O W BLAND and D S RUSSELL, *J Path & Bact* **47** 291, 1938

A series of 106 surgical specimens of dural meningioma has been analyzed. Five histologic types have been identified and described. A series of 14 tumors representative of these types has been submitted to tissue culture. In each type a great variety of cell form and growth pattern has been observed, and no cultural characteristics have been found to belong to any one histologic type of tumor. The cultural characters of meningioma have been compared with those of fetal leptomeninges and dura and adult granulation tissue. While the cultures of all the tissues studied have the features of cultures of fibroblasts, those of fetal leptomeninges differ, as a rule, from those of meningioma in not uncommonly forming epithelioid but not true epithelial sheets. This tendency toward sheet formation is considered to support the theory of the arachnoid origin of dural meningioma. A striking resemblance was found between cultures of meningioma, fetal leptomeninges and fetal dura, on one hand, and cultures of human fetal mesenchyme, on the other, both showing great polymorphism. A similar polymorphism is shown by the endothelioid cells of the leptomeninges in pathologic conditions other than neoplasm. They may form macrophages which store dyes or engulf particulate matter, and they may form fibroblasts with fibroglial fibrils. It is concluded that the leptomeninges are composed of cells which approximate to the undifferentiated mesenchymal cells with embryonic potentialities of the polyblastic system of MAXIMOW.

FROM AUTHORS' SUMMARY

RETICULOSIS AND RETICULOSARCOMA A H T ROBB-SMITH, *J Path & Bact* **47** 457, 1938

A classification of the progressive hyperplasias and neoplasias of lymphoreticular tissue is suggested which follows MAXIMOW's hypothesis of the pluripotency of certain primitive cells of the embryonic mesenchyme, which persist throughout life. The justification for a methodologic consideration of the lymphadenopathies has been put forward in the introduction. On such a framework a clinical analysis can be superimposed and a search made for etiologic factors. Further subdivisions may be necessary, or certain of those put forward may prove superfluous, but with classification based on cytologic aspects and structure such adjustments are possible without destroying the main concept, and it is only by uniformity of description and a scrupulous use of terms that any advance can be made.

FROM AUTHOR'S SUMMARY

MORPHOLOGY OF HERPES VIRUS S NICOLAU and L KOPCOWSKA, *Ann Inst Pasteur* **60** 401, 1938

In experimental herpetic encephalitis of rabbits the nuclei of the nerve cells and of the glial cells show granular masses made up of numerous elements resembling bacilli when stained with ovalated methyl blue in glycerinated dilute alcoholic acid fuchsin solution. These bodies are believed to be colonies of herpes virus. The individual elements are 0.1 to 0.2 micron in diameter. These measurements agree with those determined by ultrafiltration and ultracentrifugation.

Similar masses are found in the cytoplasm of neurons, glial cells, endothelial cells of the capillaries and monocytic cells of meningeal or parenchymatous infiltrations. After inoculation of herpes virus into the scarified cornea of the rabbit similar bodies are seen in the nuclei and cytoplasm of epithelial cells of the cornea. After inoculation of the virus into the skin of the metatarsal region of the paw intranuclear colonies are found in the malpighian cells.

In the skin of human beings suffering from herpes zoster the virus forms colonies of similar morphologic character in the nuclei of epithelial cells of the malpighian layer. After inoculation of herpes virus into a peripheral nerve many microbic cells are found between the nerve fibers, within the nerve fibers and in the axiscylinders.



Intranuclear herpetic inclusions originate from colonies of the virus by agglutination, degeneration and fusion of the organisms. The corpuscles which result are often included in a mass of the microbic cells. They gradually become eosinophilic and tend to be separated from the surrounding cell by a halo. Cytoplasmic inclusions arise in the same way and are an expression of a resistant state of the cell.

FROM AUTHORS' CONCLUSIONS

ROLE OF THE SKIN IN CHICKEN SARCOMA. A. BESREDKA and L. GROSS, *Ann Inst Pasteur* **60** 465, 1938

The incubation time of Rous sarcoma in the chicken is longer after subcutaneous than after intracutaneous inoculation. When once formed, subcutaneous sarcoma progresses rapidly to a fatal issue. Intracutaneous sarcoma appears very rapidly after injection and then progresses slowly. Intracutaneous tumors may remain stationary for months. When weak doses are inoculated intracutaneously, the tumor is resorbed in about half the cases.

Chickens which have resorbed their intracutaneous tumors become refractory to subsequent subcutaneous or intramuscular inoculations. This specific, lasting and solid immunity is of a cellular nature and is not passively transferable to other animals.

FROM AUTHORS' CONCLUSIONS

MALIGNANT TUMORS FOLLOWING PROLONGED ADMINISTRATION OF ESTROGENIC HORMONES. A. LACASSAGNE, *Bull Assoc franç p l'étude du cancer* **27** 96, 1938

This is a statistical analysis of data collected during six years, concerning which several preliminary reports have been published since 1932. Selected strains of mice, some with a high and some with a low incidence of mammary adenocarcinoma in females, some known to be very rarely afflicted with spontaneous malignant tumors, were given, beginning soon after birth, weekly injections of estrogenic hormones. Mammary adenocarcinoma was observed only in strains normally subject to it, but in larger numbers and earlier than normally, and in males as well as in females. A varying number of epithelial and connective tissue tumors appeared in strains normally known to be free from such tumors. Lacassagne concludes that estrogenic hormones played a part in the production of the tumors.

I. DAVIDSOHN

GENERALIZED LYMPHOSARCOMA OF MORE THAN NINE YEARS' DURATION. J. DUCUING, O. MILETZKY and BASSAL, *Bull Assoc franç p l'étude du cancer* **27** 117, 1938

In 1928 a lymphosarcoma, the diagnosis of which was confirmed by several biopsies, was seen for the first time in a 54 year old woman who complained of edema of the right leg. A mass was present in the right iliac fossa. After that until May 1937 the patient was examined at irregular intervals, with findings of generalized enlargement of the lymph nodes in the neck, axillae, groins and mediastinum and one breast. The spleen and later the liver were markedly enlarged. The findings varied, depending on the roentgen therapy, the dosage and the dates of administration are recorded. There was moderate to marked anemia, with periods of return to normal, and leukopenia with relative lymphocytosis, but on one occasion relative lymphopenia was recorded. Marrow obtained by sternal puncture revealed a relative decrease of granulocytes and of their precursors. The response to roentgen therapy was prompt and continued through the entire thus far observed course of the disease.

I. DAVIDSOHN

### Technical

BLOOD SUBGROUPS A<sub>1</sub> AND A<sub>2</sub>. I. DAVIDSOHN, *J. A. M. A.* **112** 713, 1939

Clinical experience indicates (1) that the selection of a donor according to known methods does not assure absence of blood transfusion reactions. (2) that

unexpected reactions are not uncommon when a donor of the same blood group as that of the patient is employed, especially when the group is O or A, and (3) that reactions are particularly frequent when so-called universal donors are employed

Available serologic data suggest (1) that subgroups  $A_1$  and  $A_2$ ,  $A_1B$  and  $A_2B$  are not always compatible, (2) that subgroups  $A_2$  and  $A_2B$  are not infrequently mistaken for other groups, particularly for O and B, and (3) that some transfusion reactions, even fatal ones, are well explained by these circumstances

The method presented offers two advantages: 1. The high-titered, easily produced and highly specific rabbit immune serum permits prompt recognition of blood group A and AB, including the feebly agglutinating subgroups  $A_2$  and  $A_2B$ . 2. A proper dilution of the serum, as determined by titration, makes it possible to differentiate subgroup  $A_2$  from  $A_1$  and  $A_2B$  from  $A_1B$  without delay. Both procedures can be carried out within five minutes.

FROM AUTHOR'S SUMMARY

DETECTION OF GLUTATHIONE IN THE MITOCHONDRIA AND NUCLEUS P. JOYET-LAVERGNE *Compt. rend. Soc. de biol.* **128** 59, 1938

The author submits a staining method for the demonstration of glutathione in the cells of plant or animal tissues. It is based on treatment of the sections with a 1 per cent cadmium lactate, converting the glutathione into a cadmium compound optically visible under the microscope. Most of the intracellular glutathione is situated in the mitochondria and nucleus of the cells.

R. J. LEBOWITZ

CHANGES OCCURRING IN BLOOD PRESERVED DURING MANY WEEKS AND THE THERAPEUTIC EFFECT OF SUCH BLOOD II. GNOISKY, *Sang* **12** 820, 1938

Gnoisky studied the properties of preserved blood obtained from 40 dogs and 80 human beings and the effect of transfusion of such blood into 18 dogs and 6 patients. The bloods were obtained aseptically, mixed with one-fifth their volume of 6 per cent citrate solution, and placed in large sterile ampules, which were hermetically sealed after all the air had been evacuated. Portions of blood were also distributed in small ampules, which were opened at intervals for testing. All specimens were kept at 4°C. A gradual decrease in the number of red cells was observed, 15 per cent of the cells having disappeared after seventy-three days. Of the red cells surviving after ninety days, 40 per cent exhibited morphologic changes. Dog blood behaved like human blood except that the cells were more fragile. The leukocytes underwent more rapid changes than the red cells, and the lymphocytes persisted longer than the polymorphonuclears. The platelets disintegrated most rapidly, completely disappearing within a period of fifteen to twenty days. Transfusions of 150 to 280 cc of stored blood into 6 patients gave rise in each case to a chill and a rise in temperature, but these were evanescent and not serious. This reaction was usually followed by an increase in the urobilinogen and the appearance of traces of hemoglobin in the urine. Nevertheless, in most of the cases there was a satisfactory rise in hemoglobin within a week.

A. S. WIENER

MAGNETIC ELECTRON MICROSCOPE AND ITS USE IN BIOLOGY F. KRAUSE, *Naturwissenschaften* **25** 817, 1937

Krause has traced the development and discussed the theoretic basis and resolving power of the Knoll-Ruska type of electron microscope. The limits of resolution are indicated. The article is illustrated with electronic photographs of cells and bacteria. The optical image sometimes differs from the electronic image, owing to differences in diffractive qualities of the two types of rays. Several methods of using the electron microscope in biology are considered and suggested.

R. J. LEBOWITZ

# Society Transactions

## BUFFALO PATHOLOGICAL SOCIETY

ERNEST WITEBSKY, *President*

Jan 28, 1939

SAMUEL SANES, *Secretary*

### CHORIOANGIOMA OF THE PLACENTA LOUIS A SIEGEL and EMERSON HOLLEY

A white American housewife, 32 years of age, gravid for the second time, was admitted in labor to the Children's Hospital, June 4, 1938. Examination disclosed a right occipitoanterior presentation and position, by rectal examination the cervix was felt to be half dilated.

Two hours after admission the membranes were ruptured artificially, a live normal boy was delivered spontaneously. The placenta and membranes were expelled completely, following use of a modified Crede technic.

The placenta was of average normal size and shape, but situated on the fetal surface near the free margin was a mass the size of a hen's egg. It measured 9 by 6 by 4 cm. The tumor was well defined, solid and firm and could be readily enucleated from the surrounding placental tissue.

Microscopic examination of the tumor showed it to be composed of a sparse amount of connective tissue stroma and an abundance of capillary-sized spaces which were lined by endothelial cells of a rather typical nature. Many of the spaces were empty, others contained red blood cells. In still other spaces remnants of hemolyzed red cells were seen. The tumor was divided into lobules by trabeculae of somewhat compressed, chiefly collagenous connective tissue, and surrounding the tumor and separating it from the placenta was a zone of compact fibrous tissue which formed a pseudocapsule.

The histologic diagnosis was chorioangioma of the placenta.

This report will be published in full in the *American Journal of Obstetrics and Gynecology*.

### THE DEVELOPMENT OF AGGLUTININS IN CHILDREN WITH DYSENTERY DUE TO SHIGELLA PARADYSENTERIAE FLEXNER ERWIN NETER

The serodiagnosis of infections due to *Shigella paradysenteriae* Flexner is considerably more difficult than that of typhoid fever or of dysentery caused by *Shigella dysenteriae* Shiga. In the first place, *Shigella paradysenteriae* Flexner comprises a number of antigenically different types including *Shigella paradysenteriae* V, W, X, Y and Z of Andrewes and Inman and other types described by Kruse and his co-workers, Boyd, Sartorius and Reploh. Second, the titer of normal agglutinins for members of the Flexner group shows marked variation and may be as high as 1:500 and above. Third, it has been reported by some authors that specific agglutinins may fail to develop in patients with dysentery caused by the Flexner organism. Finally, patients suffering from infections with *Shigella alkalescens* (Andrewes) may acquire immune agglutinins for certain types of *S. paradysenteriae* Flexner because of common antigenic components present in both species (Neter, E., and Gilfillan, R. S. *Am J Hyg* 29:67, 1939).

During the summer of 1938 23 patients with the clinical symptoms and signs of acute dysentery were admitted to the Children's Hospital. One of the patients showed marked signs of meningeal irritation, with rigidity of the neck, Kernig's

phenomenon and other signs and resembled a patient with the type of bacillary dysentery which was described by Felsen (*Am J Path* 12 395, 1936). All the patients recovered. One patient had cystitis due to *S. paradysenteriae* (Neter, E, and Fisher, W J. *Urol & Cutan Rev* 42 810, 1938). Dysentery bacilli could be isolated from the feces of 17 of these 23 patients. The micro-organisms belonged to the Flexner group. They fermented dextrose and mannitol, with production of acid but not gas, they failed to attack lactose, sucrose, rhamnose, xylose and dulcitol. The dysentery bacilli isolated from 15 patients were of the W type of Andrews and Imman, the micro-organisms isolated from the remaining 2 patients were of the Y type. Of the patients infected with *S. paradysenteriae* W, 8 were followed with respect to the development of specific agglutinins. Twenty-eight normal serums and serums of patients with diseases other than dysentery failed to agglutinate this particular type strain in titers above 1:25. In contradistinction, 7 of the 8 serums from patients with dysentery showed agglutinins in titers ranging from 1:80 to 1:1,280. In addition, the agglutination was manifest after only fifteen minutes to two hours' incubation at 55 C and was considerably stronger than that obtained with normal serums. High titers of agglutinins were observed on the seventh to fifteenth day after the onset of the illness. In several cases serum specimens were tested during the first five days of illness and found to lack agglutinins for the strain of *S. paradysenteriae* which was isolated from the patients. In one particular case, for instance, the serum obtained on the third day of the disease had an agglutinin titer of less than 1:10, while the serum obtained on the seventh day of the illness strongly agglutinated the strain under investigation in a dilution of 1:80. One patient presenting the clinical signs of dysentery was never found to have dysentery bacilli in the feces, the development of specific agglutinins during the course of the illness in this particular patient gives strong evidence as to the cause of the disease, particularly since this child was 1 of 3 siblings in all of whom dysentery developed.

It may be concluded from these observations that in outbreaks of bacillary dysentery, as presented in this article, the examination of the serum of the patients for immune agglutinins for the homologous micro-organisms may support and supplement the bacteriologic findings.

#### PANCREATOGENOUS STEATORRHEA IN AN INFANT S. SAINES and RICHARD A. DOWNY

At birth a white boy weighed 6 pounds 15 ounces (3147 Gm). He was the first born. In the second week of life he began to have three to twelve stools per day. Some of these were loose and watery. Most were large, soft, yellow and foul. Fat in the stool was 51 per cent of the dry weight. Diets prescribed for their constipating effect brought no relief from the frequent large stools. When the boy was 3 months old, signs of a respiratory infection developed. The abdomen also became distended. Despite the fact that he took his feedings well, he remained the same weight as at birth until his death, at 7½ months of age.

The anatomic diagnosis was pancreatogenous steatorrhea, marked marasmus, anemia, hyperplasia of the bone marrow, marked purulent bronchitis and bronchiolitis with bronchiectatic abscesses and chronic interstitial pneumonia and bronchopneumonia, atypical metaplasia of the epithelium of bronchioles, pulmonary emphysema, gross atresia of the cystic duct with glandular formations in the wall containing inspissated secretion, slight rickets, hyperemia with hemorrhage in the medulla of the adrenal, digested milklike content in the trachea and bronchi, hyperemia and hemosiderosis of the spleen, hemosiderosis of the liver.

Microscopically, the lobular structure of the pancreas could still be discerned. The acinar tissue was not well preserved. The ducts were dilated. They contained detritus, apparently secretion, desquamated cells and few neutrophils. Fat was demonstrated in the ductular content, particularly within large cells. There was distinct interlobular and intralobular inflammatory reaction, with infiltration of round cells, plasma cells, eosinophils and neutrophils and marked fibrosis. Hemosiderin was revealed free and in macrophages. The capillaries were dilated.

Several medium-sized arteries showed hyaline thickening of the internal elastic lamina. The walls of these vessels as well as of smaller arteries seemed somewhat thickened. Islets of Langerhans were present in good numbers. Some were hypertrophic. Bacteria were not found with special stains.

The observations in our case correspond to those summarized by Anderson and Blackfan in their recent publications. We interpret the lesion in the pancreas in the light of obstructive cirrhosis.

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## PATHOLOGICAL SOCIETY OF PHILADELPHIA

BAXTER L. CRAWFORD, *President*

*Regular Meeting, Jan 12, 1939*

H. L. RATCLIFFE, *Secretary*

### ALEUKEMIC PLASMA CELL LEUKEMIA WITH HEMORRHAGIC PHENOMENA MITCHELL BERNSTEIN and DAVID FISHBACK

Aleukemic plasma cell leukemia is rare. Its occurrence with hemorrhagic phenomena is still more unusual. Our patient, aged 52, complained of bleeding from the gums and marked weakness when he first came under observation, on Feb 2, 1938. The bleeding from the gums began in November 1937 and occurred almost daily.

The striking features were pallor, hemorrhages from the gums, petechiae in the buccal membrane, a soft apical systolic cardiac murmur, enlargement of the spleen to 8 cm below the left costal margin, and a few petechiae over the thighs. Marked secondary anemia was found, the hemoglobin content being 27 per cent, the erythrocyte count 1,750,000 and the leukocyte count 3,500 per cubic millimeter, with 60 per cent neutrophils, 37 per cent lymphocytes and 3 per cent monocytes. The platelets averaged 110,000 per cubic millimeter. This blood picture did not change with subsequent examinations. The bleeding time was six and one-half minutes, and the coagulation time was three and one-half minutes. Chemical examination of the blood revealed hyperproteinemia, reversal of the serum albumin-globulin ratio and a low cholesterol content. An unusual feature of the blood was its peculiar tendency to gel on withdrawal from the patient's vein regardless of the addition of citrate or oxalate.

The urine contained albumin, hyaline casts and occasionally double refractile bodies. Routine studies of the gastrointestinal tract showed a normal condition. Roentgen studies of the skeleton revealed nothing of interest. The sternal bone marrow during life was said to be hypoplastic.

From February 2 on, the patient had repeated oozing hemorrhages from the gums and nose. On May 18 bilateral thrombosis of the retinal veins occurred, and weakness, pallor and splenic enlargement persisted. Slight enlargement of the liver was noted. Broncho pneumonia finally developed, and death occurred on June 22.

At necropsy the features of interest were splenomegaly, marked myocardial degeneration, infarction of the left lung, confluent bronchopneumonia, nephrosis and nephrosclerosis, fatty metamorphosis of the liver, with slight congestion, and hyperemia of the brain. Histologic examination (Dr R. P. Custer) showed various tissues, especially the spleen, bone marrow, lymph nodes, liver and adrenals, to contain large collections of plasma cells. Plasma cells were also noted within a small blood vessel.

# DISSECTING ANEURYSMS SIMULATING SYPHILITIC HEART DILATASI, WITH AORTIC REGURGITATION E. O. ANDERSON and B. A. GOUIN

A study of autopsy records at the Philadelphia General Hospital during the past ten years has disclosed a total of 40 cases of dissecting aneurysm—on an incidence of 1 in 400. In 16 of the 40 cases the classic signs and symptoms of rapid dissection were presented with death due to external massive hemorrhage. In 6 of the 40 cases there were signs and symptoms of aortic regurgitation. Death was due not to rupture of the aneurysm and extravasation of blood but to long-standing heart failure. At autopsy the cross section of the aorta usually presented an onion peel appearance. The dissection of the media frequently extended the entire length of the aorta, from the root to or beyond the bifurcation of the iliac arteries. In 2 cases the dissection remained within the confines of the media, there having been neither internal or external rupture. In the other cases the dissection communicated with the true aortic lumen both proximally and distally, producing a double barrel channel. There was no separation of the commissures but the aortic ring was dilated, and the central portions of the free margins of the aortic cusps were moderately rolled and thickened so as apparently to prevent adequate approximation. The heart was invariably hypertrophied. In 3 additional, similar cases, the dissection had not extended proximally to the root of the aorta or had not existed long enough to cause signs and symptoms of aortic regurgitation, and these instances were therefore excluded from this series.

The clinical diagnosis in the majority of the cases was syphilitic heart disease with aortic regurgitation. Even at autopsy in 4 of the 6 cases the gross anatomic diagnosis was syphilitic aortitis. However, in no case could syphilitic aortitis or any other syphilitic entity be substantiated by microscopic examination.

## RECURRENT BENIGN PROSTATIC HYPERTROPHY ASSOCIATED WITH CARCINOMA W. P. JENNINGS

According to available information, the incidence of recurrent benign hypertrophy of the prostate is approximately 2 per cent. From a brief review of reports it appears that the development of carcinoma of the prostate in association with recurrent hypertrophy is much less frequent. Two examples of the latter condition are presented here. The ages at which both the first and the second phase of the disease occurred are within the expectable range. The patients were recently admitted to the Presbyterian Hospital, to the services of Dr. George C. Griffith and Dr. Joseph C. Birdsall, respectively.

CASE 1—A white man 71 years old, was admitted to the hospital Nov. 9, 1938, because of hematuria of five weeks' duration. In June 1933 an enlarged prostate had been removed, which showed adenofibromatous hypertrophy and chronic prostatitis. The patient's convalescence was uneventful. In June 1937 he noted increasing constipation. From July 1938 to the date of admission he lost 50 pounds (22.7 Kg.) in weight and experienced weakness of the arms and legs, dyspnea and intercostal neuralgia. In October 1938 hematuria was noted, and the testes were swollen and painful. Proctoscopic and cystoscopic examination apparently were not informative. The blood pressure was elevated. There was marked tenderness over the left side of the abdomen, as well as slight tenderness over the right, chiefly over an enlarged liver. The scrotum was swollen and inflamed, with edema of the scrotum and on the left side, and the left testis was enlarged and soft. The rectal sphincter was spastic, and a large hard mass could be palpated on the anterior rectal wall. The urine contained a trace of albumin and numerous leukocytes. The blood count was normal. The patient died on November 22. At autopsy the immediate cause of death was demonstrated to be a coronary occlusion with infarction of the interventricular septum. The bladder was trabeculated. The urethral mucosal surface was smooth and white, with two firm white sessile nodules, 3 and 2 cm. in diameter, respectively, bulging from the lateral surfaces of the otherwise smooth prostatic bed producing no obstruction. Periurethrally in the prostatic region was a mass of firm white tissue 6 by 5 by 3 by 2 cm.

within which were numerous punctate yellow areas and minute, extremely hard white elevations. The main portion of this extended from the urethra to the rectum. The testes and penis were apparently normal. Microscopically, the superficial tissues in the prostatic urethra contained a mass of prostatic elements showing benign hypertrophy. A few hyperplastic ingrowths were present. However, the underlying muscular coats and periurethral tissues were infiltrated by neoplastic cells, among which few mitotic figures were seen. Chronic inflammatory changes were associated with this process. Distal to the prostatic tissue, the urethral mucosa was ulcerated, and the superficial as well as the deep fasciae were infiltrated with tumor cells. The diagnosis was primary adenocarcinoma of the prostate, grade 3, and recurrent benign hypertrophy. Metastases were not found.

CASE 2—A white man aged 82 years was admitted to the hospital on Nov 29, 1938, because of increasing frequency of urination of two weeks' duration, resulting in acute retention on the morning of admission. Transient hematuria was noted in October 1938. In September 1917 an enlarged and indurated prostate had been removed. Convalescence was uneventful. At the present admission the results of physical examination were essentially negative, there was, however, slight cardiac enlargement, and the prostate was large, irregular and hard. Cystoscopic examination revealed marked trabecular hypertrophy and what were apparently diverticula, there was a prominent anterior notch with protruding intravesical, lateral and median lobes. On December 14 a large prostatic mass, 7 by 5 by 3 cm, and four smaller masses, each 1 cm in diameter, collectively weighing 95 Gm, were removed. The prostatic tissues were well encapsulated, had a honeycomb appearance and were composed of multiple yellowish white masses of varied size in a soft fibrous matrix. The gross impression was recurrent benign hypertrophy. Microscopically, sections through seven portions of the gland were examined, all of which showed bulky, often greatly dilated glands lined by hyperplastic columnar epithelium. In several areas, however, the epithelium assumed a distinct neoplastic character and was definitely invasive. Tumor cells were noted in a dilated lymphatic channel. The diagnosis was recurrent benign prostatic hypertrophy and adenocarcinoma of the prostate.

#### EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF THE CHICK EMBRYO LAWRENCE E W SMITH

The effect of changes in temperature on the development of the chick embryo was found to be profound in a series of over 700 instances. A critical temperature lying somewhere between 90 and 95 F exists at which not only delay in development was noted but also failure of cell growth, differentiation and organization. The significant fact was observed that the first forty-eight to ninety-six hours were the most important. Eggs which at the beginning of their development were subjected to a temperature of 90 F for forty-eight or more hours and then allowed to complete their incubation at a normal temperature (102-103 F) showed 100 per cent malformations. These ranged from minor defects of the extremities to actual monstrosity. Ectopia cordis, eventration, sirens and even double-headed monsters were obtained with absolute regularity. Temperatures well below this critical level resulted merely in delayed maturation. Temperatures above 105 or 106 F caused rapid disintegration of the embryo.

These results point toward a basic biologic principle in relation to cell growth. Its possible application as a therapeutic adjunct in the treatment of inoperable, advanced cancer, dependent on the idea of a critical level of temperature for young, actively growing tumor cells, is suggested. Preliminary clinical observations in a small group of cases seem to bear out the soundness of this idea.

#### "SILENT," SO-CALLED PRIMARY TUBERCULOSIS OF THE SPLEEN HERBERT FOX

Three cases are reported in which so-called primary tuberculosis of the spleen was discovered by splenectomy. The condition is called primary because no other

locality of disease could be unexceptionally diagnosed. The term "silent" is suggested because only the splenomegaly called attention to the condition. Tubercle bacilli were sought for by all the accepted methods without success, and this has been the experience of others. Tuberculosis of the spleen has been found associated with purpura, pernicious anemia, hemolytic icterus, leukemia, polycythemia and Banti's syndrome. The silent form is usually fibromiliary, the caseous form is associated with more easily discoverable tuberculosis. The tuberculin test is unreliable. This chronic fibromiliary form seems never to have been diagnosed before operation.

The article will be published in full in the *American Journal of Clinical Pathology*.

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BANTLER L. CRAWFORD, *President*

*Regular Meeting, Feb 9, 1939*

H. L. RATCHIFF, *Secretary*

#### MURAL ENDOCARDITIS FOLLOWING PULMONARY SUPPURATION. A. TRASOFF and D. R. MIRANZE

A woman aged 47 years became suddenly ill with symptoms and signs suggestive of acute pancreatitis. These continued for two weeks, and then the condition followed a more septic and toxic course.

The physical signs were those of an infection of the upper respiratory tract and pneumonitis of the lower lobe of the right lung, with marked abdominal distention and epigastric tenderness. The blood studies all gave negative results except for anemia. The urine showed a trace of albumin.

Within two weeks after the onset a large spleen was palpable, and examinations of the blood, urine and feces for typhoid bacilli and brucellas revealed none. A roentgenogram of the lungs revealed marked congestion but no pneumonia. The disease progressed rapidly, the temperature becoming more septic, finally meningeal involvement became apparent. The patient died before a lumbar puncture could be obtained.

On postmortem examination were found a large spleen, which contained a hemorrhagic cyst, a mural thrombus in the left auricle of the heart, a large abscess in the upper lobe of the left lung and smaller abscesses in the upper lobe of the right lung with unresolved pneumonia. Abscesses were also found in the liver, kidney, perirenal fat tissue and brain, from which a type III pneumococcus was isolated. The pancreas showed definite evidence of acute interstitial pancreatitis, as noted by marked edema and infiltration.

#### BENIGN ENLARGEMENT OF THE PROSTATE. ROBERT A. MOORE

Benign enlargement or benign hypertrophy of the prostate occurs commonly in the presenile and senile periods of life. In postmortem examinations there is found a progressive increase in the incidence with increase in age, so that men in the ninth decade show an incidence of 75 per cent. In clinical examinations the greatest incidence is found in persons in the seventh decade of life, with fewer cases in both younger and older persons. The disease also shows varying incidence in some races and is more frequent in married than single men.

The earliest lesion is found in the periurethral tissue and consists of focal hyperplasia of the stroma. If glands are in immediate contact with the foci in the stroma, there is simultaneous hyperplasia of the epithelium. Only that part of the prostate is involved in which the ducts empty into the urethra above the lower end of the verumontanum. This part of the prostate is represented in the female pseudohermaphrodite. The prostate thus consists of two parts, a male part below



and an ambisexual part above the verumontanum. The same separation can be established in a study of the prostate of a newborn infant which has been exposed to a large amount of estrogen in the mother's blood.

On the basis of these facts and others it seems probable that the estrogenic hormone in the male organism plays a role in the genesis of benign enlargement. My own studies for the past two years have been directed toward development and proof of this working hypothesis.

It is well known that androgens will neutralize the effect of estrogens if injected in a ratio of about 50 to 1. It is also known that there is a gradual decrease in the urinary excretion of androgens with increasing age. It follows that if the amount of effective androgen decreased more than the estrogen during presenility the prostate would be exposed to unneutralized estrogen. In an analysis of 60 specimens of urine from patients with and without benign enlargement there is evidence to support this assumption.

Studies of the morphologic aspects and hormone content of the pituitary gland in benign hypertrophy eliminate this organ as the primary seat of the disease. The absence of the disease in true eunuchs lends strong support to a theory that the most probable cause is a disturbance in the hormone secretion of the testes.

The most satisfactory working hypothesis is: Benign enlargement of the prostate is a disease of presenility due to the altered and irregular differential secretion of androgen and estrogen during this period of life.

## Book Reviews

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**La maladie D'Aujeszky** P Remlinger, directeur de l'Institut Pasteur de Tanger et J Bailly chef de service a l'Institut Pasteur de Tanger. Papei Pp 204 with 16 illustrations. Price 45 francs. Paris. Masson & Cie, 1938

This is the only monograph on a disease known under several names, 'infectious bulbar paralysis, 'mad itch,' 'pseudorabies,' but known best under the name of its discoverer, who described it in 1903 in Hungary, as a previously unknown disease in dogs, cats and cattle. In 1912 the disease was observed in Brazil, later in various European countries, then in Africa and Asia, and in 1930 it was found by Shope in the state of Iowa. A biographic introduction deals with the life of the discoverer, Adar Aujeszky, 1869-1933. Then follow chapters on the history of the recognition of the disease all over the world, the symptoms it gives rise to in the different species and the complicated epidemiologic problems which it presents. While the disease has been found in only a limited number of animals, all of them mammals, it can be reproduced experimentally in a large number of species, among them all the usual warm-blooded laboratory animals, and by practically all the known routes of infection. Important observations on the experimental aspects of the disease are found in the third chapter. In man the disease has been observed in a few isolated instances, and its course was mild. The peculiarity of most of the affected animals that they die of the disease at night is the subject of a special chapter. The experimental transmission of the disease, the characteristic posture of the dead animals, which resemble stuffed museum specimens, the scratch lesions, the self-inflicted mutilations due to extreme itching, the secondary infections, the rather insignificant changes in the internal organs, the inflammatory lesions in the central nervous system and the changes in the urine and blood are described in chapter 6. The cause of the disease, a filterable virus, is present in practically all tissues of the body and absent from most of the secretions and excretions. The nature of the virus and its relation to the virus of rabies and of herpes are considered. The diagnosis and the treatment, what there is of it, are discussed, and good reasons are presented in favor of using the experimental forms of the disease for teaching of virus diseases in medical schools. An extensive bibliography, which includes thirty publications by the authors, concludes the monograph. This work ought to be of great interest to veterinarians and to students of virus diseases.

**The Biology of Bacteria. An Introduction to General Microbiology**  
Second edition. Arthur T. Henrici, Professor of Bacteriology, University of Minnesota. Pp. viii + 479 and index. Price \$3.60. Boston. D. C. Heath & Co., 1939.

That a second edition of Dr. Henrici's text on general microbiology should appear is in itself a guaranty of merit, that it should appear so soon after publication of the first edition indicates not only a continuing growth in the subject matter but also a realization that the science of bacteriology can be well served only by making its discoveries and the newer interpretations based thereon available to the present day student. Textbooks in such an active field must inevitably be somewhat behind the frontier, but surely both the science and the scientist deserve a frequent sharpening of their tools. This is provided in this treatment of the basic phases of bacteriology. Individual chapters have been revised, and additional chapters have been introduced, to the end that a more complete and a broader point of view may be presented to the student. The text meets the purpose admirably.

The author is to be commended for acting on the very evident fact that today no single course in bacteriology can meet all needs, no single author or teacher of bacteriology can be competent in all fields and that no single textbook of bacteriology adapted to practical use by the student can treat authoritatively all phases of the subject. An introductory course based on this text will provide an excellent point of departure for advanced study in many fields.

## Books Received

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RECENT ADVANCES IN FORENSIC MEDICINE Sidney Smith, M S, F R C P D P H, Regius Professor of Forensic Medicine, University of Edinburgh, and John Glaister, M D, D Sc, J P, of the Inner Temple, Barrister-at-Law, Regius Professor of Forensic Medicine, University of Glasgow Second edition Cloth Pp 264, with 85 illustrations Price \$4 50 Philadelphia P Blakiston's Son & Co, 1939

COLLECTED PAPERS OF THE MAYO CLINIC AND THE MAYO FOUNDATION Edited by Richard M Hewitt, B A, M A M D Volume XXX, 1938 Cloth Pp 1065, with 149 illustrations Price \$11 50 Philadelphia and London W B Saunders Company, 1939

THE HARVEY LECTURES Delivered under the Auspices of the Harvey Society of New York, 1938-1939 Under the Patronage of the New York Academy of Medicine Dr Guy F Marrian, Dr A Ashley Weech Dr Eugene F du Bois, Dr Edwin J Cohn, Dr Edwards A Park, Dr K Linderstrøm-Lang, Dr C H Danforth, Dr Albert Szent-Gyorgyi Series XXXIV Cloth Pp 279, with illustrations Price \$4 Baltimore Williams & Wilkins Company 1939

BLOOD GROUPS IN AFRICA Ronald Elsdon-Dew, M D Publications of the South African Institute for Medical Research, Edited by the Director No XLIV (Vol IX, pp 29-94) Paper Johannesburg, South Africa The South African Institute for Medical Research, 1939

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## CORRECTION

In the review by Drs Weil, Gall and Wieder entitled "Progress in the Study of the Typhoid Bacillus," in the July issue of the ARCHIVES (28 71, 1939), reference was made on page 78 to a statement by Drs Delafield and Smith regarding the effect of somatic antigen on the oxygen uptake of organs The June issue of the *British Journal of Experimental Pathology* (20 216, 1939), however, contains a later report by these authors, stating that the effect was due to some material which was present in the trypsin used in the preparation of the antigen, and not to the antigen itself

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## TRANSPOSITION OF THE GREAT CARDIAC VESSELS

WITH SPECIAL REFERENCE TO THE PHYLOGENETIC  
THEORY OF SPITZER

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Transposition of the great vessels has attracted attention as "the most interesting and puzzling" of congenital cardiac malformations. Other defects, such as persistent foramen ovale, patent ductus arteriosus, abnormal aortic arches, may be considered as mere developmental defects, i. e., as evidences of the persistence of certain stages in embryonic development. In transposition, however, a radical departure from normal development has apparently taken place, to the great disadvantage of the organism. Similar arrangements cannot be compatible with normal complete development, for at no point in ontogeny are

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similar forms definitely seen. A number of ingenious hypotheses have been advanced in explanation. It is the purpose of this paper to offer a summary of them. Since a complete exposition of the phylogenetic theory of Spitzer has never appeared in English literature, his work will be examined in greater detail. To illustrate this theory, examples from the collection in the Children's Hospital will be cited. Excellent accounts of the normal embryology of the heart are readily available, and the reader is referred to them for review (Davis, Monckeberg, 1924, Keibel and Mall, 1910).

#### GLOSSARY

There has been considerable variation in the use of many of the terms by different writers. It is therefore deemed advisable to list definitions of the terms as they appear in this paper.

*Bulboatrial ledge* The projecting ledge in the primitive heart cavity caused by the bulboatrial cleft.

*Conus arteriosus* The smooth-walled anteromedial portion of the right ventricle which gives rise to the pulmonary artery.

*Crista supraventricularis* The thick rounded muscular ridge, averaging 12 to 15 mm in height, which arches across the roof of the right ventricle from the ventricular septum to the anterolateral ventricular wall. It lies behind the pulmonary artery and in front of the anterior cusp of the tricuspid valve. Between the valve and the crista there is a fossa of varying depth. This is illustrated in figure 5, page 9, of Abbott's "Atlas of Congenital Cardiac Disease."

*Detorsion* The term used by Spitzer to designate a deficiency in the normal twisting of the entire heart in an early period of development.

*Dextrocardia* The location of the heart in the right side of the thoracic cavity, with the apex pointing toward the right. It seems preferable to refer to types of dextrocardia as intrinsic or extrinsic, depending on whether the condition is due to causes within or to causes outside of the heart. This would obviate the necessity for the term "dextroversion" as used by Lichtman to designate extrinsic dextrocardia.

*Dextroversion* According to Spitzer, the type of functionally corrected transposition in which the aorta arises left anterior from the left-sided ventricle, and the pulmonary artery from the right posterior portion of the right-sided ventricle. The atrioventricular valves are usually inverted. (See case 16.) The term has been used by others to signify an extrinsic congenital dextrocardia (Paltauf, Monckeberg).

*Dextroposition of the aorta* The insertion of the aorta to the right of its normal position. Increasing degrees of dextroposition carry the aorta first to the riding position over the interventricular septum, then into the midportion of the right ventricle and finally to an anterior position in the same ventricle.

*Heart of reptiles* The sinus venosus lies on the dorsal wall of the atrium and varies considerably in size in the different species. The atria are divided into right and left chambers by a complete septum. Two atrioventricular orifices are present. The ventricular cavity is divided by an incomplete septum which runs from the ventral wall to the dorsal portion of the right lateral wall. The ventricles are therefore situated right ventrally and left dorsally. Both atria open into the dorsal chamber, which gives off two separate aortas. The pulmonary artery arises from the ventral chamber. The dorsal chamber is subdivided by a trabecular formation.

which runs from the back of the first septum to the dorsal wall of the ventricle. This formation passes between the two aortas and inserts between the atrial orifices. In crocodiles it forms a new complete ventricular septum, while the first septum undergoes regression to form the *Muskelleiste* (muscle ledge) of Greil. The latter separates the pulmonary artery from the right aorta. The three great vessels all contain bicuspid valves. "It appears that the ventricular septum in the Crocodilia (and in the hearts of birds and mammals) consists of two parts, the one of which, the anterior part, represents the division of the ventricle to effect a separation of the outgoing blood, and the other, the posterior part, the division to maintain the separation of the incoming atrial blood. The former of these parts appears first, and apparently is that found generally throughout the reptilia, while the latter remains small and is largely replaced by the atrio-ventricular valves" (Walmsley, 1929)<sup>1</sup>

*Infundibulum*    Synonymous with conus arteriosus

*Inversion*    Also called situs inversus in contrast to the normal situs solitus. The formation of an organ in right left mirror picture to that of the normal without alterations in the anteroposterior relationships.

*Moderator band*    A trabecular formation, variable in extent and form in man, which stretches across the lower part of the right ventricular cavity from the lower third of the ventricular septum to the anterolateral ventricular wall in close relation to the anterior papillary muscle of the tricuspid.

*Muskelleiste of Greil*    See "Heart of Reptiles"

*Septum aorticopulmonale*    The portion of the truncus bulbus septum which separates the pulmonary artery from the two aortas in reptiles and from the single aorta in mammals.

*Septum aorticum*    The portion of the truncus bulbus septum which separates the left ventricular aorta from the pulmonary artery (and the right ventricular aorta in reptiles).

*Sinus of the right ventricle*    The posterior or inflow portion of the right ventricle, which is separated from the infundibular, or outflow, portion by the crista supraventricularis and the trabecula septomarginalis. This demarcation is more clearly defined in the contracted heart and is shown distinctly in figure 39 in Tandler's "Anatomie des Herzens."

*Trabecula septomarginalis* } The trabecula septomarginalis is commonly held  
*Tricuspid ledge* } to be identical with the moderator band as defined  
in a foregoing paragraph. Spitzer has expressed the belief, however, that the structure is a composite made up of the trabecula proper and the apical portion of the anterior tricuspid ledge. The former, together with the crista supraventricularis, forms the inferior aorticopulmonary septum (crista aorticopulmonalis). Originally the structure had the function of separating the pulmonary artery from the right ventricular aorta and is therefore homologous to the *Muskelleiste* of Greil. The second part, the apical portion of the anterior tricuspid ledge, comprises the anterior papillary muscle of the tricuspid valve, the medial muscle of Lancisi and the posterior portion of the moderator band between these structures. Because of the obliteration of the right ventricular aorta, the apical portion of the tricuspid ledge has become fused to the posterior border of the trabecula proper.

<sup>1</sup> Several definitions (e. g., those of crista supraventricularis and moderator band) follow closely those given by Walmsley in Quain's "Elements of Anatomy." See bibliography, under "general references."

The superior, or basal, portion of the anterior tricuspid ledge is composed of the anterior attachment of the tricuspid ring and the anterior leaflet with its chordae. It is separated from the crista supraventricularis by the niche which marks the site of the obliterated right ventricular aorta. The fusion of the tricuspid ledge and the crista supraventricularis to form the composite trabecula septomarginalis forms the Y-shaped ledge on the interventricular septum, referred to by Humphreys. The medial and posterior tricuspid leaflets with their chordae, papillary muscles and basal attachments are derivatives of the posterior tricuspid ledge.

*Transposition* Abbott, in Osler's "Modern Medicine," has defined transposition as "an alteration in the position of the two great vessels relative to the ventricles of the heart, or to each other at their origin, so that they either spring from reversed ventricles, the aorta from the right, and the pulmonary artery from the left chamber (complete transposition), or from the ventricle to which they normally belong, but in reversed relationship (corrected transposition)." In certain cases, however, the definition of complete transposition must be supplemented by the words *and an alteration in the anteroposterior relationships of the great vessels, either at the ventricular insertion or in their splicing*. Otherwise cases of pure inversion could be referred to as transposition, e. g., the position of the great vessels in complete situs inversus of the entire body. Furthermore, the term "ventricle to which they normally belong" may refer, in the case of the pulmonary artery, either to the tricuspid-bearing chamber, or the right-sided chamber regardless of structure, or to the systemic venous ventricle. Since these meanings may connote different ventricles in the same heart, it appears advisable to use the term "corrected" in a functional sense only. Thus a corrected transposition would be one in which the pulmonary artery arose from the ventricle receiving the systemic venous blood and the aorta from that receiving oxygenated pulmonary blood.

#### HISTORICAL INTRODUCTION

The first description of a series of cardiac abnormalities was given by Senac in 1749. His efforts to explain their pathogenesis were futile, and he refers to them as the manifestations of an "intelligence formatrice." In 1812 Meckel sought to explain various anomalies on an embryologic basis and came to the conclusion that they were *Hemmungsbilden* (failures of development at an embryologic stage).

Two years later Farie, a clinician, discussed the various forms of congenital heart disease in brilliant fashion. He classified them physiologically into defects causing mingling of the blood and defects causing impediment to the circulation. He also called attention to the frequent occurrence of the triad of pulmonic stenosis, overriding aorta and interventricular septal defect. It is interesting to note that Farie defended his departure to the field of pathology by stating that only from the pathologic basis could the picture of congenital heart disease be understood. The wisdom of this statement has been amply demonstrated by the fundamental work of Abbott, who in this manner was successful in bringing order and comprehension into the chaotic field of cardiac anomalies. In 1837 Kurschner stated that transposition occurred because of failure in the splicing of the great vessels and incorrect fusion of the septums of the ventricle and the truncus. Meyer

came to the same conclusion in 1857, after studying a case of complete transposition "Embryology furnishes an easy means of explaining the transpositions from faulty development, that is, from an arrested development of the great vessels in an embryological stage in which the spiral torsion of the vessels had not yet taken place" No other comment is made

The best early work on the subject in English was published by Peacock in 1858 His classification was similar to that of Meckel Defects were arranged according to the points of time in intrauterine life at which arrest in development was assumed to have taken place Peacock was well in advance of his time and his work still remains an excellent source of clinical and pathologic material He recognized, with Farie, the frequent occurrence of the combination of pulmonary stenosis, overriding aorta and ventricular septal defect He pointed out the long duration of life in this condition and demonstrated that in this triad the patency of the foramen ovale and the ventricular septum allowed life to continue for longer periods In regard to complete transposition he stated "cases of this kind afford examples of complex deviations consisting partly in arrest of growth at early periods of fetal life, and partly in irregularity in the evolution of the main arteries, the aorta and pulmonary arteries, from the single arterial trunk and the branchial arches" Very shrewd were his comments concerning comparative anatomy He stated that the usual interventricular septal defect between the subaortic region of the left ventricle and the sinus of the right ventricle was homologous with the foramen between the two aortic ventricles of the turtle This view was upheld in 1922 by Abbott and Shanly, who showed that a defect between the left ventricle and the right ventricular sinus was comparable to the interventricular foramen between the left and right halves of the dorsal ventricle of the turtle Finally Peacock foreshadowed the work of Spitzer in the statement that "the sinus and infundibular portions of the right ventricle in man are the analogues of the right systemic and pulmonic ventricles of the turtle" "In the former case" (of transposition in which both the aorta and the pulmonary artery arise from the right ventricle) "the analogy to the form of the heart in the *Chelonia* is most decided, the aorta arising from the one cavity—the sinus of the ventricle—and the pulmonary artery arising from the other—the infundibular portion of conus arteriosus" In certain cases of transposition (see fig 6, type 4, page 453) he noted that the connection of both atria with the left ventricle might be ascribed to the right ventricle having been completely divided by a septum at the point of union between the sinus and infundibular portions This assumes regression of the true interventricular septum These observations, made seventy-five years ago, are essentially in accord with the recent theory of Spitzer



Later writers, up to the time of Rokitansky, busied themselves with describing malformations and discussing the theories concerning the origin of the combination of interventricular septal defects, pulmonary stenosis and overriding aorta. Morgagni, in 1761, had suggested that primary pulmonary stenosis might hinder the blood flow from the right ventricle and thus cause the other defects (obstruction or *Stauung* theory). Meckel held, however, that the interventricular defect was primary and that the pulmonic stenosis resulted from insufficient blood flow (which we shall call prepulmonic deviation of blood). Heine, in 1861, came to the conclusion that primary deviation of the ventricular septum to the left caused overriding of the aorta, persistence of the septal defect and finally pulmonic stenosis (through prepulmonic deviation). Halbertsma decided on coincidental primary movement of the truncus septum to the right and of the ventricular septum to the left, so that both pulmonary stenosis and overriding by the aorta arose simultaneously.

In 1865 Kussmaul reviewed all previous theories and stated that the growth of the ventricular septum must take place before the growth of the truncus septum and the spiraling of the aorta and pulmonary arteries. Both vessels originate from the ascending limb of the primitive ventricular loop which is continued into the common truncus and which later forms the right ventricle. The overfilling of the right ventricle by blood coming through a primary ventricular defect would cause deviation of the ventricular septum to the left and make union with the truncus septum impossible. In this manner the aorta would come to the overriding position with its lumen in relation to both ventricles.

#### THEORY OF ROKITANSKY

Such was the status of knowledge of congenital heart disease when Rokitansky, in 1875, published the "Defecte der Scheidewande des Herzens." In this work a comprehensive scheme of pathogenesis of cardiac anomalies is based directly on embryologic studies. Only that portion which concerns abnormalities in the position of the great vessels will be discussed here.<sup>2</sup>

Rokitansky postulated that the normal truncus septum begins as a swelling in the left posterior portion of the primitive truncus. This swelling grows toward the right anterior portion in a curved fashion, with the convexity normally directed anteriorly. Rokitansky believed that the curvature determined the nature and position of the vessels, the aorta being always on the concave side and the pulmonary artery always on the convex. Thus the normal aorta was thought to be in the right posterior position, and the pulmonary artery in the left anterior position, at the insertion into the ventricles. The septum ventriculorum arises

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<sup>2</sup> Excellent discussions of the entire theory of Rokitansky are to be found in the works of Assmus, Herxheimer and Rauchfuss.

to the left of the common truncus, so that both vessels at first open into the right ventricle. A defect in the anterior portion of the ventricular septum, however, affords an opening for the aorta into the left ventricle. The aorta is soon transferred completely into the left ventricle by the growth of the right side of the ventricular septum around the right base of the aorta toward the concave inferior edge of the truncus septum and by the fusion of these septums with the right end of the endocardial cushions of the atrioventricular canal.

Rokitansky postulated, on teratologic grounds, that the form of the ventricle depends on the position of the ventricular septum. The ventricle on that side from which the septum ventriculorum takes origin will be arranged as the normal left ventricle with a bicuspid valve, while the other will have the conus arteriosus, heavy trabeculation and tricuspid valve characteristic of the normal right ventricle. To explain why the truncus and ventricular septums develop normally from the left side, Rokitansky hazarded the guess that they were influenced by the origin of the descending aorta and ductus arteriosus (normally on the left side).

It is to be noted that Rokitansky saw no reason for assuming a close correlation between the spiraling of the great vessels and the direction of the lower truncus septum. On that account he considered only the lower end of the truncus in his theory of the origin of transposition. The stress thus laid on the faulty anlage and development of the truncus septum constitutes, according to Monckeberg, one of Rokitansky's most important contributions.

On this conception of embryonic development is based his explanation of cardiac malformations. The truncus septum may assume any position in the lower truncus. The septum ventriculorum may therefore vary in its inception either from the right or from the left. This gives eight possible variations in the Rokitansky scheme, doubled by the assumption that the septum may have the concavity reversed. (See fig 1.) Note that in series A 5 to 8 and series B 5 to 8 the septum takes origin from the right, so that the character of the ventricles is transposed. Series A represents "corrected transposition" since the aorta arises from the left ventricle and the pulmonary artery arises from the right ventricle as normally. Scheme B represents transposition (uncorrected) in situs solitus. The "correct" (*gehorige*) ventricle for Rokitansky in situs solitus was the left for the aorta and the right for the pulmonary artery—even though that left ventricle might on occasion have a tricuspid valve (as in series A 5 to 8). Furthermore, because isolated inversion of the atria was then unknown, Rokitansky's corrected anatomic transposition was also corrected functionally.

For cases of pulmonic and aortic stenosis Rokitansky postulated an eccentric position of the truncus septum. The diagram is self-explanatory (fig 1, series D).

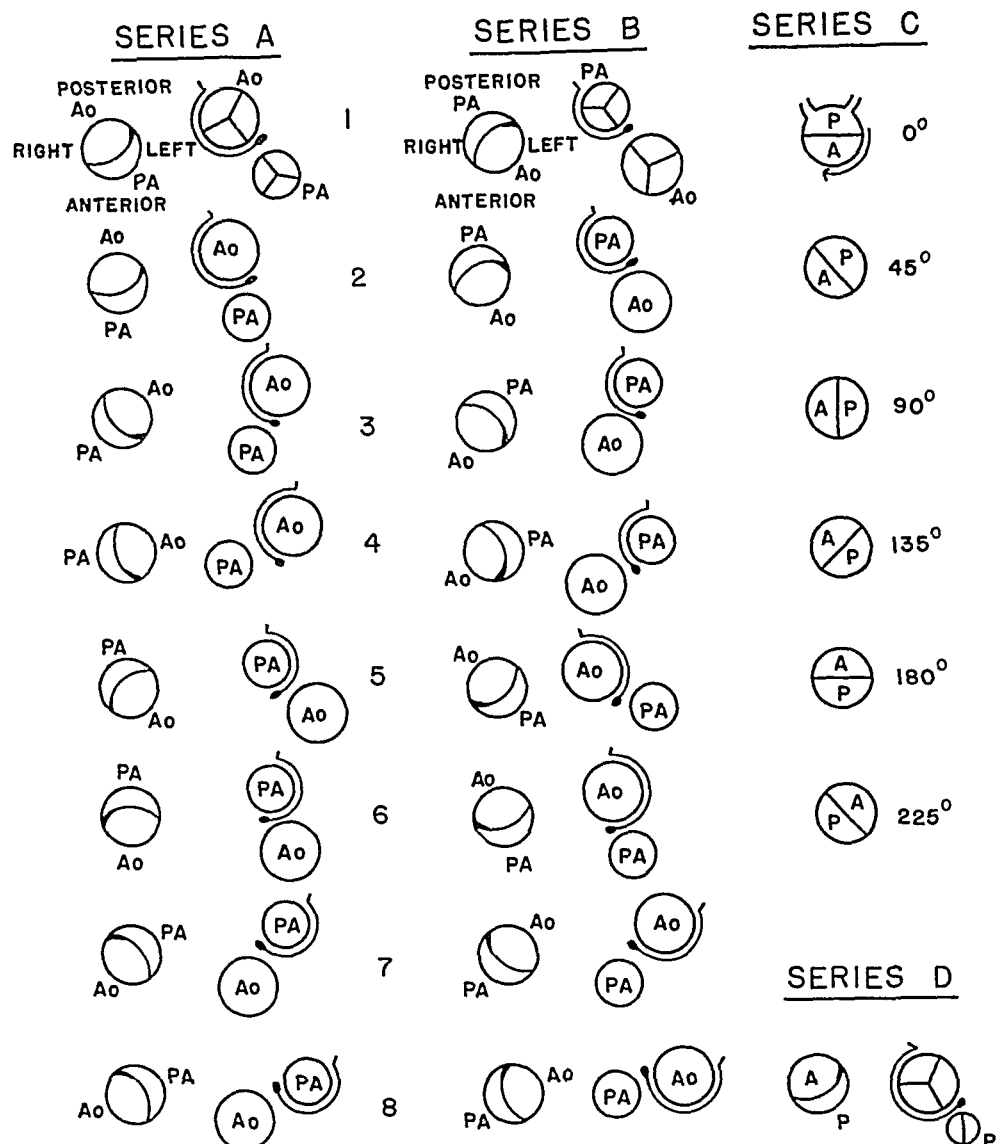


Fig 1—Series A illustrates the various forms of corrected transposition and series B the forms of uncorrected transposition (both after Rokitansky). The left hand column of each series shows the position of the truncus septum in the undivided truncus arteriosus. The rounded end represents the point of origin of the septum. The right hand column of each series shows the condition of the interventricular septum after the separation of the great trunks. The rounded end corresponds in position with the point of origin of the truncus septum. The hooked end represents the membranous portion of the septum. The structures are viewed from above and anteriorly. Diagram A 1 shows the normal condition. Ao indicates the aorta and Pa the pulmonary artery.

Series C illustrates the normal spiraling of the truncus septum. The truncus has been sectioned at various levels from the point of origin of the sixth branchial arch above to the level of the insertion of the great vessels into the ventricles below.

Series D illustrates the origin of pulmonary stenosis in an eccentric position of the truncus septum as postulated by Rokitansky.

## MODIFICATIONS OF THE THEORY OF ROKITANSKY

The weaknesses inherent in the Rokitansky theory were quickly discovered, and revisions were made. Born showed that the normal position of the aorta is left posterior, and that that of the pulmonary artery is right anterior, at the ventricular insertion. It was discovered that the truncus septum arises from the growth of two opposing pairs of swellings (distal bulbar swellings I and III and proximal bulbar swellings *a* and *b*). Lochte pointed out that the determination of the vessel destined to be the aorta or the pulmonary artery depends not on the concavity of the truncus septum but on the position of the vessels at the division of the truncus into the sixth branchial arch and the spiral form of the truncus septum. Aschoff showed that the aorta is normally directly anterior, and the pulmonary artery directly posterior, at the level of the distal portion of the truncus. It is then clear that in order to reach the Born position at the ventricles, the truncus septum must undergo a clockwise spiral of 225 degrees toward the heart (fig 1, series C). When the separation of the vessels is completed, the normal twisting of the pulmonary artery around the aorta is evolved. Transposition may then be explained by a deficient degree of spiraling. If this is limited to 45 degrees, the vessels are transposed, and the aorta arises in right anterior, and the pulmonary artery in left posterior, position. In *situs inversus* a counterclockwise torsion normally takes place, and limitation of this to 45 degrees will cause the origin of the aorta to be placed in the left anterior portion, i. e., in the position of transposition in *situs inversus*. These are the two basic forms of transposition.

Other hypotheses have been formulated to account for certain forms of transposition. Lewis and Abbott studied a heart in which a large ventrally placed aorta emerged from a small, imperfectly separated portion of the ventricle and crossed in front of the pulmonary artery. The latter arose posteriorly, in the position of a normal aorta, from the main portion of the ventricle. The remainder of the heart was essentially normal. Lewis and Abbott concluded that a bending of the original cardiac tube to the left, instead of to the right, brought the great vessels into an apparently reversed relationship. The anterior aorta was then displaced to the right to join the right ventricle.

Many years before, Keith had postulated a similar occurrence of a reversed bend of the ventricular loop to account for transposition, but Stokes had objected that in such an event there should be an antero-posterior interchange of the aorta and pulmonary artery. Since this is not always true, Stokes then assumed that the anomaly arose because of faulty spiraling of the truncus septum. In the case of Abbott and Lewis there is an actual anteroposterior interchange of the great vessels, but it is difficult to understand how a reversed bend of the cardiac

tube can affect only the trunks and allow the remainder of the heart to form normally. One would logically expect the architecture of the ventricles to be inverted.

#### CORRECTED TRANSPOSITION

In 1894 Lochte pointed out that the position of the great vessels in corrected transposition in situs solitus is identical with that in ordinary transposition in situs inversus (aorta left anterior and pulmonary artery right posterior). Likewise the position in corrected transposition in situs inversus is the same as that in the usual transposition in situs solitus. Lochte therefore believed that an inversion of the bulboventricular loop coupled with the usual type of transposition was the cause of corrected transposition. A corrected transposition in situs solitus should therefore show situs solitus of the atria, situs inversus of the bulboventricular loop, inversion of the atrioventricular orifices and limitation of the counterclockwise (inverted) torsion of the truncus septum to 45 degrees.

Geipel, however, strongly opposed this conception, returning to the principles of Rokitansky and to one of Lochte's earlier postulates. A bending of the original cardiac loop (normally to the right) in the opposite direction would place the descending portion on the right and the ascending part on the left. The ascending, or truncus, portion would thus be taken from the right into the future left ventricle. Finally the posterior vessel, the pulmonary artery in this event, would be transferred from the left to the right ventricle. Normally the posterior vessel is the aorta, and it is transferred from the right to the left ventricle (see page 433). It is with the direction of this transfer that Geipel correlated the formation of the atrioventricular valves, the mitral valve always arising on the side to which the transfer of the posterior vessel is made. In corrected transposition, therefore, according to Geipel, the mitral valve should be in the right ventricle. He opposed Lochte's assumption of a true situs inversus of the ventricles on the grounds that in corrected transposition in situs solitus the left sixth branchial arch forms the ductus arteriosus as usual, the position of the coronary arteries is normal, and the outer muscular layer of the heart retains the normal structure. Geipel, however, was in error in the three objections. It has since been shown that either the left or the right sixth arch may form the ductus arteriosus. Spitzer in 1923 demonstrated that the coronary arteries may be transposed regardless of the situs of the ventricles, and Taussig showed that only the two inner muscular coats of the heart are reversed in true complete situs inversus. The direction of the outer layer remains as in situs solitus. Furthermore Monckeberg demonstrated an actual inversion of the ventricles in certain cases of corrected transposition by showing that the character of the atrioventricular bundles was interchanged. Lochte's assumption

of an inversion of the bulboventricular loop in corrected transposition is accordingly correct

Geipel classified the types of transposition as follows

A Pure transposition

- 1 Situs solitus I<sup>3</sup>
- 2 Situs transversus<sup>1</sup> VIII
- 3 Situs solitus and transposition of the ventricles II
- 4 Situs transversus<sup>4</sup> and transposition of the ventricles VII

B Corrected transposition

- 1 Situs solitus III
- 2 Situs transversus<sup>1</sup> VI
- 3 Situs solitus and transposition of the ventricles IV
- 4 Situs transversus<sup>1</sup> and transposition of the ventricles V

It will be noticed that neither Lochte's nor Geipel's theories can explain the rare cases of A 3 and 4 and B 1 and 2. Lochte stated that there may have been errors in the observations in the reported cases, while Geipel regarded them as merely "inexplicable variations of nature not to be contained in a cold scheme." Monckeberg, however, explained these by a clockwise or a counterclockwise spiral of the truncus septum—in other words, by the possibility of an isolated situs solitus or situs inversus of the truncus segment of the heart. For example, in type B 1, he assumed a situs solitus of all of the heart save the truncus segment, the septum of which undergoes a counterclockwise torsion of 45 degrees. Spitzer expressed the belief that isolated inversions of any portion of the heart may occur (in the bulbar, ventricular or sinoatrial regions). Any one of these may be combined with a transposition and thus give rise to corrected transposition, either functional or anatomic.

The case reported by Walmsley may be cited as an example of isolated inversion. The heart showed corrected transposition of the arterial stems. The aorta arose left anteriorly and the pulmonary artery right posteriorly. Inversion of the ventricles and situs solitus of the atria were present. Walmsley stated that a true inversion of the ventricles with a 180 degree counterclockwise spiral of the proximal portions of the great vessels had occurred, and that this spiral was partially canceled by a 90 degree clockwise spiral of the upper part of the uninverted truncus septum.<sup>5</sup> Other cases are seen in which a total

3 The Roman numerals in the classification refer to the diagrams in figure 2

4 This term corresponds to "situs inversus"

5 The summation of these two spirals would be a 90 degree counterclockwise twisting of the great vessels. In consideration of the variety of types (A 3, A 4, B 1 and B 2) in which the situs of the bulbus and ventricles differ, it seems more probable that a situs inversus of the bulboventricular loop coupled with a limitation of the counterclockwise spiral to 90 degrees gave rise to the condition found

inversion of the body and atria is coupled with the solitus position of the ventricles

#### INVERSION

Monckeberg and Spitzer pointed out that inversion is the result of a mirror picture anlage and has little to do with instances of transposition in which the defect probably appears later in the course of development. The heart in complete situs inversus is a mirror picture of the normal. One of the manifestations of transposition is a disturbance in the anteroposterior relationships of the great vessels, the aorta usually assuming a more anterior position either in the left or in the right ventricle. Thus, in corrected transposition, regardless of the presence or absence of inversion, the transposition is definitely

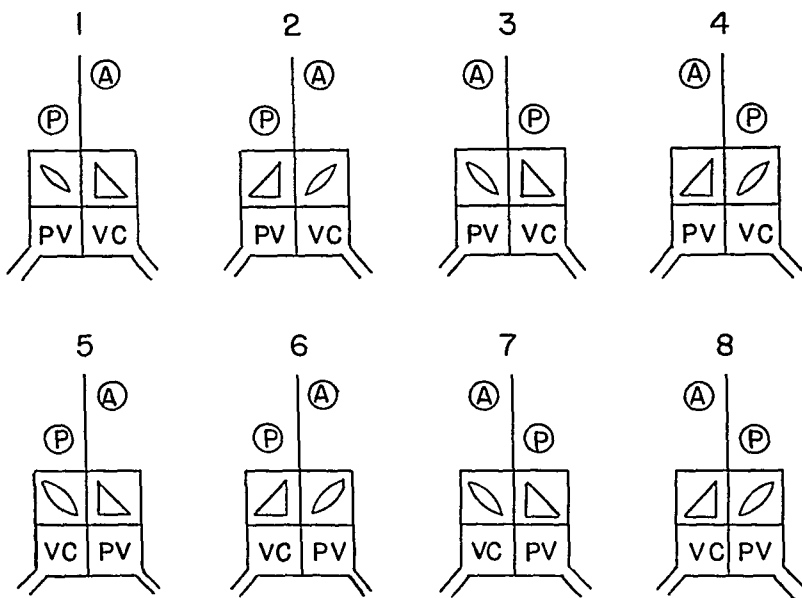


Fig 2 —Diagrams illustrating the various types of complete transposition of the great vessels. *A* indicates the aorta, *P*, the pulmonary artery, *PV*, the pulmonary veins, and *VC*, the vena cava. The triangle represents the tricuspid valve, and the ellipse represents the bicuspid valve.

According to the nomenclature of Spitzer 1 is pure crossed transposition in situs solitus, 2, crossed transposition with ventricular inversion, 3, crossed transposition with bulbus inversion, 4, crossed transposition with ventricular and bulbar inversion, 5, crossed transposition with sinoatrial inversion, 6, crossed transposition with sinoatrial and ventricular inversion, 7, crossed transposition with sinoatrial and bulbar inversion, and 8, inverse crossed transposition.

According to the nomenclature of Monckeberg 1 is pure transposition without ventricular transposition (situs solitus), 2, pure transposition with ventricular transposition in situs solitus, 3, corrected transposition without ventricular transposition in situs solitus, 4, corrected transposition with ventricular transposition in situs solitus, 5, corrected transposition with ventricular transposition in situs inversus, 6, corrected transposition without ventricular transposition in situs inversus, 7, pure transposition with ventricular transposition in situs inversus, and 8, pure transposition without ventricular transposition in situs inversus.

established by the anterior shift of the aorta, and the posterior shift of the pulmonary artery, in relation to the ventricles. Nevertheless, although inversion and transposition appear to be distinct and individual disturbances, the frequency of the combination of partial inversion and transposition (called dextroversion by Spitzer) is very much greater than would be expected.<sup>6</sup> Other puzzling aspects arise—the essential nature of an inversion, the mode of origin and the manner in which an inverted portion of the heart joins with the normally developed parts. Interesting light is thrown on the subject by the work of Stohr, who demonstrated normal development of the amphibian heart both when transplanted and when turned 180 degrees about the transverse axis. To consider these questions, however, is not within the scope of this paper.

Pernkopf and Wirtinger recently proposed a theory of the cause of transposition founded on the concept of inversion. In 1933 they reported an exhaustive study of normal cardiac development based on the assumption that the dorsal mesocardium marks, at all times, the original longitudinal axis of the primitive cardiac tube (isogonal diameter). This fixed position serves as a guide to the torsions which take place during cardiac development. They also determined the roles of the two halves (antimeres) of the original tube through a study of the stage when complete fusion had not as yet taken place.

From their study Pernkopf and Wirtinger concluded that the septal formations arise in a spiral form. There is a 180 degree counterclockwise turn of the septal anlage in the sinoatrial region. A clockwise turn of 180 degrees is present in the ventricular portion, and another 180 degree clockwise rotation is present in the bulbar region. The total spiral of the septal anlage is 180 degrees, and the systemic and pulmonic circuits therefore cross—allowing systemic venous blood to enter the pulmonary arteries and oxygenated blood to pass out through the aorta. To these spirals are added the movements of the cardiac tube during two phases of development.<sup>7</sup> Briefly, the first phase consists of a clockwise twist of the atrial portion of the primitive tube. The torsion reaches its maximum of 90 degrees at the site of the future atrioventricular junction. At the same time the bulbar portion undergoes a counterclockwise rotation of 90 degrees which reaches its peak at the region of the bulboventricular junction. Since the tube is

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6 In fact, only a single case has been reported in which partial situs inversus of the bulboventricular portion with situs solitus of the atria has not been accompanied by transposition of the great vessels (Ratner, B., Abbott, M. E., and Beattie, W. W. *Am J Dis Child* **22** 508, 1921).

7 Although the appearance of the septums actually occurs after completion of the first phase, it has been described first for easier comprehension.



fixed at both ends, the middle or ventricular portion must show a total rotation of 180 degrees in a counterclockwise direction. In the second phase, a counterclockwise twist of 150 degrees occurs at the bulbo-ventricular region and a 45 degree diminution of the phase I torsion at the ventriculobulbar junction takes place. The final form of the heart is thus evolved.

Table 1, which is taken from Pernkopf and Wirtinger, shows the form of the septums and the torsions of the cardiac wall in the phases of development.

Several important points are apparent in table 1. First, the crossing over of the pulmonic and systemic circuits is seen to result only from the inherent spiraling of the septum and not from any hydrodynamic factors. Second, the total effect of any torsion of the cardiac tube must always be zero, since the tube is fixed at both ends. For the same reason, the total spiral of the normal septums must always be 180 degrees.

TABLE 1—*Form of the Septums and Torsions of the Cardiac Wall in Phases of Heart Development*

Segment of Heart	Original Form of the Cardiac Septums	Phase 1 Movement of Cardiac Wall	Form of Septums After Phase 1	Phase 2 Movement of Cardiac Wall	Final Form of the Septums
Truncus	0°	0	0	+150°	+150°
Bulbus	+180°	+ 90	+270	-150°-45°	+ 75°
Ventricular	+180°	-180°	0	+ 45°	+ 45°
Sinoatrial	-180	+ 90	- 90°	0°	- 90°
Total	+180°	0°	+180	0°	+180°

in a clockwise direction. Third, any variation in the movements of the cardiac wall may change the form of the heart but cannot alter the crossing over of the two circuits. The latter function resides solely in the spiral nature of the septal anlage.

On these foundations Pernkopf and Wirtinger erected their theory of partial inversion as the cause of transposition. Since each cardiac segment originally contains a 180 degree spiraled portion of the septum, the complete inversion of any segment will not alter the crossing over of the pulmonic and systemic circuits (obviously it makes little difference whether the blood takes a clockwise or a counterclockwise turn in any segment as long as the total of 180 degrees is achieved). On the other hand, failure of the septum in any segment of the heart to show a spiral would result in complete separation of the two circuits such as exists in the usual form of transposition. The blood would then take a straight or 360 degree spiraled course to return to the same circuit it left. Pernkopf and Wirtinger noted that the possibility of partial inversion arises in each of the three segments because the septum reaches the anterior and posterior walls in the midportion of each sec-

tion (forming points of bilateral symmetry) They explained the usual transposition as the result of partial inversion of the septal anlage in the bulbar region They assumed that the proximal portion of the bulbar septal anlage in this condition shows a clockwise turn of 90 degrees while the distal portion shows a counterclockwise (inverted) twist of 90 degrees The total spiral in that segment is therefore zero Since the remainder of the heart is normal, the total spiral of the entire septum is also zero, and the pulmonary and systemic circuits do not cross Pernkopf and Wirtinger assumed that similar partially inverted "septa transponans" may arise in the other cardiac segments and thus give rise to the various forms of corrected transposition as shown in table 2

TABLE 2—*Form of Septum Anlage in Various Types of Complete Transposition (According to Pernkopf and Wirtinger)*

Type in fig. 2	Crossed Transposition		Functionally Corrected Transposition		Functionally and Anatomically Corrected Transposition		Anatomically Corrected Transposition	
	In Situs Solitus	In Situs Inversus	In Situs Solitus	In Situs Inversus	In Situs Solitus	In Situs Inversus	In Situs Solitus	In Situs Inversus
	1	8	5	4	6	3	2	7
Bulbar region	Inverse (-90°)	Normal (+90°)	Inverse (-90°)	Normal (+90°)	Inverse (-90°)	Normal (+90°)	Inverse (-90°)	Normal (+90°)
	Normal (+90°)	Inverse (-90°)	Normal (+90°)	Inverse (-90°)	Normal (+90°)	Inverse (-90°)	Normal (+90°)	Inverse (-90°)
Ventricular region	Normal (+90°)	Inverse (-90°)	Normal (+90°)	Inverse (-90°)	Normal (+90°)	Inverse (-90°)	Normal (+90°)	Inverse (-90°)
	Normal (+90°)	Inverse (-90°)	Normal (+90°)	Inverse (-90°)	Inverse (-90°)	Normal (+90°)	Inverse (-90°)	Normal (+90°)
Sinoatrial region	Normal (-90°)	Inverse (+90°)	Normal (-90°)	Inverse (+90°)	Inverse (+90°)	Normal (-90°)	Inverse (+90°)	Normal (-90°)
	Normal (-90°)	Inverse (+90°)	Normal (+90°)	Inverse (-90°)	(+90°)	(-90°)	Normal (-90°)	Inverse (+90°)
Total spiral of septum	0	0°	+180°	-180°	+180	-180°	0°	0°

Through the close relationship of total and partial inversion, Pernkopf and Wirtinger explained the frequent occurrence of the combination of inversion and corrected transposition They also stated that the *invariable* presence of transposition in hearts showing a different situs of the atria and ventricles is strong confirmation of the hypothesis that partial inversion is the cause of transposition (Pernkopf and Wirtinger, 1935) The previously cited case of Ratner, Abbott and Beattie, however, shows the combination of situs inversus of the bulbo-ventricular loop and situs solitus of the atria without the presence of transposition of the great vessels

Recently, Lev and Saphir criticized the theory of Pernkopf and Wirtinger on the ground that it is difficult to conceive of cardiac development without the influence of irritative hydrodynamic factors While discarding the idea of an inherent spiraled nature of the septum forma-

tion, they retained the concept of the movements of the cardiac wall during ontogenesis. After a careful study of 6 cases of transposition, they advanced the theory that the fundamental disturbance in cases of transportation is faulty absorption of the bulbus. Normally, according to Pernkopf and Wirtinger, there is a 270 degree clockwise spiral of the bulbar septum after the completion of phase I. In an attempt to undo this excessive spiral there is normally a 150 degree torsion at the ostium bulbotruncate, with a back torsion of 45 degrees at the ostium ventriculobulbare. These torsions are intimately associated with absorption of the bulbus. Lev and Saphir assumed that if this absorption cannot proceed normally, the excessive bulbar torsion may be overcome abnormally by increased back torsion at the ostium ventriculobulbare together with decreased torsion at the ostium bulbotruncate. In this manner, that portion destined to become the aorta will be shifted from its normal position over the left ventricle to a position over the right ventricle, and a transposition will result.

#### EARLY PHYLOGENETIC THEORIES

The study of phylogeny has gradually thrown light on the causes of transposition. Keith, studying the fate of the bulbus cordis through phylogeny, assumed that originally the pulmonary artery led into the left side of the bulbus and the aorta into the right. He postulated that a normal expansion of the left side of the bulbus formed the conus and pushed this structure anteriorly to the right to join the right ventricle. The usual atrophy of the right side of the bulbus brought the aortic orifice backward and to the left. Keith argued that these changes were reversed in transposition, i. e., that the left side failed to expand sufficiently to bring the pulmonary artery to the right ventricle. Keith himself, however, remarked on the frequency with which pulmonary stenosis occurred in transposition (when the left side failed to expand). He wondered why subaortic stenosis did not occur more frequently when the right side of the bulbus atrophied as it was postulated to do under normal conditions. The theory has been discarded as incorrect, mainly because the original positions of the vessels as assumed by Keith could not be verified.

The work of Robertson on the development of the bulbar valves in dipnoan fishes was an important advance. She recognized that "the phenomenon, therefore, of the twisting of the great vessels must be indissolubly linked with the gradual development of a complete pulmonary respiration, and therefore also with the development of a completely four-chambered heart." She attempted to elucidate the "difficulty in explaining so marked a rotation of the arterial end of the heart without there being any evidence of similar rotation (but in an opposite direction) of the venous end of the organ" by demonstrating

that lengthening and kinking of the bulbus in dipnoan fishes can cause formation of a spiral valve from the straight ridges that are seen in the Elasmobranchii. Incidentally, she also rearranged Rokitsky's series, after pointing out that A 1 to 4 were mirror images of B 8 to 5 and likewise that A 5 to 8 were mirror images of B 4 to 1. The former could be explained by increased clockwise spiraling of the bulbus septum in *situs solitus* (A 1 to 4) or increased counterclockwise spiraling in *situs inversus* (B 8 to 5) and the latter by deficient spiraling again either in *solitus* (B 4 to 1) or *inversus* (A 5 to 8). (See fig. 1.)

#### PHYLOGENETIC THEORY OF SPITZER

The importance of phylogeny in relation to cardiac malformations became increasingly manifest in the works of Robertson and Keith. After Greil and Lange had made their exhaustive embryologic and comparative anatomic studies of reptiles and birds,<sup>8</sup> the time was ripe for a phylogenetic theory of cardiac development. In 1919 and 1921 Spitzer correlated these findings in a comprehensive theory based on the circulatory demands of pulmonary respiration and terrestrial life working through a phylogenetic series. He postulated that *the formation of the septums in the heart are teleologically conditioned, phylogenetically brought about and mechanically achieved by the appearance and development of the lungs through phylogeny*. The greater metabolic requirements of both pulmonary and systemic circuits demand that the force of the heart be brought directly into these circuits instead of in tandem form, such as exists in the fishes. Moreover, the gradually increasing circulation brings about the division of the heart through forces inherent in the blood stream.

Beneke was the first to point out some of these forces. He assumed that the whorls set up when a stream passes by a constricted portion may cause the formation of valves through a pull on the immediately distal portion. More recently Bremer analyzed the influence of the two streams coming to the heart through the venous sinuses and showed that the spiraling which occurs may explain a good deal of the torsion and septum formation. Spitzer divided the forces of the blood stream into the continuous hydraulic component, the pressure of a pulsating stream and the effects incident to increasing volume of circulation. These forces act even before the onset of pulmonary respiration but are greatly strengthened when the latter makes its appearance. At first these forces tend to stretch the original cardiac tube in all directions. Widening takes place, but since the tube is fixed at both ends, a longitudinal pull is exerted through the walls. This pull tends to draw the dividing

8 See Hochstetter for a discussion of the development of the hearts of various vertebrates.

partitions between the main cardiac vessels and their proximal branches nearer the heart, while the pressure of the two streams on each side excites such partitions to growth. In this manner Spitzer explained the gradual progression of the pulmonary arteries and veins toward the heart and the shortening of the truncus arteriosus through phylogeny. Geil also took these forces into consideration and ascribed the essential and primary means of the formation of the bulbar septum to this centripetal movement of the partitions between the vessels.

Growth and muscular invasion of the region of the future ventricle take place early in development. Contraction of this portion of the heart tube forces the blood into the immediately distal segment and distends it to form the bulbus. Meanwhile the momentary stagnation during systole widens the tube in its proximal portion and forms the atria and sinus venosus. The endocardium at the narrow junctions between these cavities is thrown into folds by the longitudinal pull associated with the widening ("much as a cloth is thrown into folds when a pull is exerted"). The continuation of the forces leads next to lengthening of the tube as well as to further dilatation, and a loop must form since the heart is fixed at both ends. In this manner the hearts of the higher fishes are formed.

With the advent of pulmonary respiration in phylogeny, a very much greater volume of blood must pass through the heart. Bending alone becomes inadequate to compensate for the lengthening tendency, and torsion must take place. The original right bend initiates the torsion to the right, and the bulbar elements are thrown into a clockwise spiral. Since the heart is fixed at both ends, detorsion must take place and a counterclockwise spiral must be present at the opposite or venous end.

According to Spitzer, this is the most important stage in cardiac development. *Without it no advance could take place, with faulty degrees of torsion the most bizarre anomalies result.* The concept of torsion recurs repeatedly through Spitzer's hypothesis, and its importance cannot be underestimated. The septum formation must not only separate the pulmonary and systemic circuits but also cross the circuits so that systemic venous blood enters the pulmonary artery and oxygenated blood passes out through the aorta. A straight septum could only cause the circuits to exist side by side as in cases of complete transposition. Torsion conditions the necessary spiral at the arterial end and thus permits the crossing over of the circuits. In order that the countertorsion may not undo this effect, the countertorsion must take place *peripheral* to the entrance of the pulmonary veins. Furthermore, it is through the torsion that the course of the longitudinal folds, along which the blood flows easily, is directed more or less into the current. The forces residing in the blood stream may then work on the folds, stimulate them to grow and cause them to develop into septums.

An examination of the formation of the atria may serve to make this clear. Since the pulmonary vein enters the heart central to the vena cava, the portions of the heart which form the left and right atria must first be placed in tandem formation. The septum primum, as the dividing partition between the pulmonary and systemic circuits, is directed sagittally across the current. The partition grows and tends to shut off the current from the vena cava. This causes pressure and marked increase of the right side of the future right atrium. The partition is forced to turn with the current and in time reaches the middle of the atrioventricular orifice. The folds *distal* to the septum primum are thrown into a counterclockwise spiral because of the counter-torsion already mentioned. These folds form the left and right valvulae

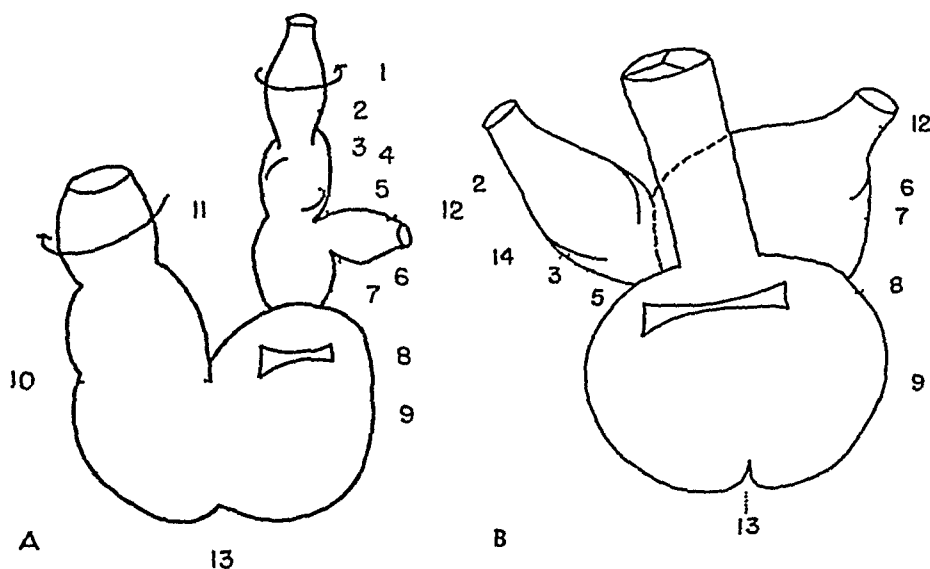


Fig 3—Development of the atrial region (after Spitzer, 1923 [c]), *A*, early stage, *B*, later stage. 1, indicates an arrow showing counterclockwise torsion of the venous end of the heart, 2, the sinus venosus, 3, the right atrium, 4, the folds which later form the septum secundum, 5, the septum primum, 6, the crista terminalis, 7, the left atrium, 8, the atrioventricular orifice, 9, the ventricle, 10, the bulboatrial ledge, 11, an arrow showing clockwise torsion of the arterial end of the heart, 12, the pulmonary vein, 13, the anlage of the septum ventriculorum, and 14, the right valvula venosa

of the vena cava, and when pressed against the septum they close the foramen ovale. According to expectations, all valves and structure distal to the pulmonary veins should show counterclockwise torsion. "A glance at the Born model<sup>9</sup> will demonstrate complete accord with this theory" (Spitzer)

<sup>9</sup> The Born model is depicted in Tandler's "Anatomie des Herzens" (fig 22, p 30)

Spitzer pointed to a number of ontogenetic facts to demonstrate the validity of the foregoing description of early cardiac development the original tube form of heart with four outpocketings in tandem formation, the right bending and right torsion of the tube, the right or clockwise spiral course of the bulbar septum and the interventricular foramen,<sup>10</sup> the left spiral of the venous valves and the shifting course of the septums. He argued that one should not expect to find the entire series of postulated events, because of the condensation of the ontogenetic recapitulation of phylogenetic events.

For an explanation of the truncus bulbus region, Spitzer turned to comparative anatomy. In reptiles, most definitely seen in crocodiles, three great vessels arise from the heart: an aorta arising from the left ventricle, an aorta arising from the right ventricle and a pulmonary artery arising from the right ventricle anterior to the right-chambered aorta, separated from the latter by a ledge, the *Muskelleiste* of Greil. From a comparison of the conditions found in reptiles, birds and man, Spitzer postulated a hypothetic original form which is now extinct. In the following discussion the truncus bulbus ventricular region will be described as though it were a straight tube, but it must be remembered that all the swellings actually retain their spiral course. For example, distal bulbar swelling I courses from the right posterior side of the bulbus at the truncus level to a left anterior position nearer the ventricles.

*Original Form* (fig 4 A) —The septum aorticipulmonale, separating distally the anteriorly placed aortas from the pulmonary artery, is postulated as beginning in the growth of the partition between the fifth and sixth aortic arches on both sides. Since the pulmonary artery is relatively small at this stage in phylogeny, the septum finally meets and joins bulbar swelling I and II in the distal bulbar region. These swellings hypertrophy and join to form the septum aorticipulmonale in the bulbar region. In a similar manner the septum aorticum, which separates the two aortas, arises from the partition between the third and fourth left aortic arches. Lower down the posterior leaf meets the septum aorticipulmonale and is deflected to the right to join distal bulbar swelling I. The fusion of distal bulbar swelling I with IV forms the remainder of the septum aorticum which encloses the left-chambered aorta.

Spitzer maintained that the formation of two septums in this fashion is a mechanical and teleologic necessity. The septum aorticipulmonale begins distally because of the separation of the pulmonary arteries from the remainder of the truncus. It divides the systemic and pulmonic arterial streams. The septum aorticum, on the contrary, develops in response to the two streams (of oxygenated and reduced blood) com-

<sup>10</sup> See Keibel and Mall on the course of the interventricular foramen.

ing from the atria. It should therefore first appear proximally. The two septums do not meet exactly, because of the spiral torsion of the truncus. The inertia of the blood causes the blood stream to undergo a slightly less spiraled course than the truncus itself. This would cause a relative counterclockwise torsion of the septum aorticum and place it at right angles to the septum aorticopulmonale.

Spitzer argued teleologically that the presence of the right ventricular aorta is necessary because of the still inadequate development of the lungs. The animal must maintain a shunt from the right ventricle to the systemic circulation in order to prevent undue pulmonary congestion, to utilize the force of the powerful right ventricle in the systemic circuit and to decrease the blood flow through the lungs during unfavorable metabolic conditions.

It will be noticed that distal bulbar swellings I, II and IV take part in the formation of septums. All four swellings form valves lower down in the bulbar region. The position of these valves is shown in figure 4. In the lower bulbar region swelling I is continued into proximal bulbar swelling *a*, while swelling IV runs into *b*. Swellings *a* and *b* form part of the septum ventriculorum, while swelling *c*, the continuation of distal bulbar swelling II, deviates laterally into the right ventricle to form the *Muskelleiste* of Greil separating the pulmonary artery from the right-chambered aorta.

*Reptilian Form* (fig. 4B).—The increase in lung capacity in these animals has resulted in a corresponding increase in the diameter of the pulmonary artery. The posterior portion of the septum aorticopulmonale is therefore forced from its original position on distal bulbar swelling II to distal bulbar swelling III. At the same time the enlargement of the sixth aortic arch encroaches on the fifth arch, and the latter atrophies. The remainder of the formations remain approximately as in the original form. According to Spitzer, the valvular portions of the swellings are not affected by the shifts of the septal parts. The valvular portions, lying flat against the vessel walls, are not subject to the forces of the changing currents, while the septal portions are easily modified, since they extend into the blood stream. In this manner the migration of a septum may split a valve in two. Each of the three great vessels in reptiles contains two valve leaflets.

*Avian Form* (fig. 4C).—With the marked increase in blood flow in this class, the pulmonary artery is greatly distended, and a corresponding increase in flow to the left ventricle and left-chambered aorta takes place. The pulmonary artery and the left aorta increase in size and obliterate between them the right-chambered aorta which is no longer needed. The divergent leaves of the two septums, fastened originally to distal bulbar swellings II and IV, are pushed over to



distal bulbar swelling III This causes obliteration of the left fourth arterial arch which was formerly the continuation of the right-chambered aorta The single aorta of birds must therefore empty into the right fourth arterial arch, as it actually does In the ventricular region the proximal bulbar swellings course as usual, save that swelling *c* undergoes regression since it is not excited to growth by the presence of two streams on either side of it Since proximal swellings *a* and *b* and

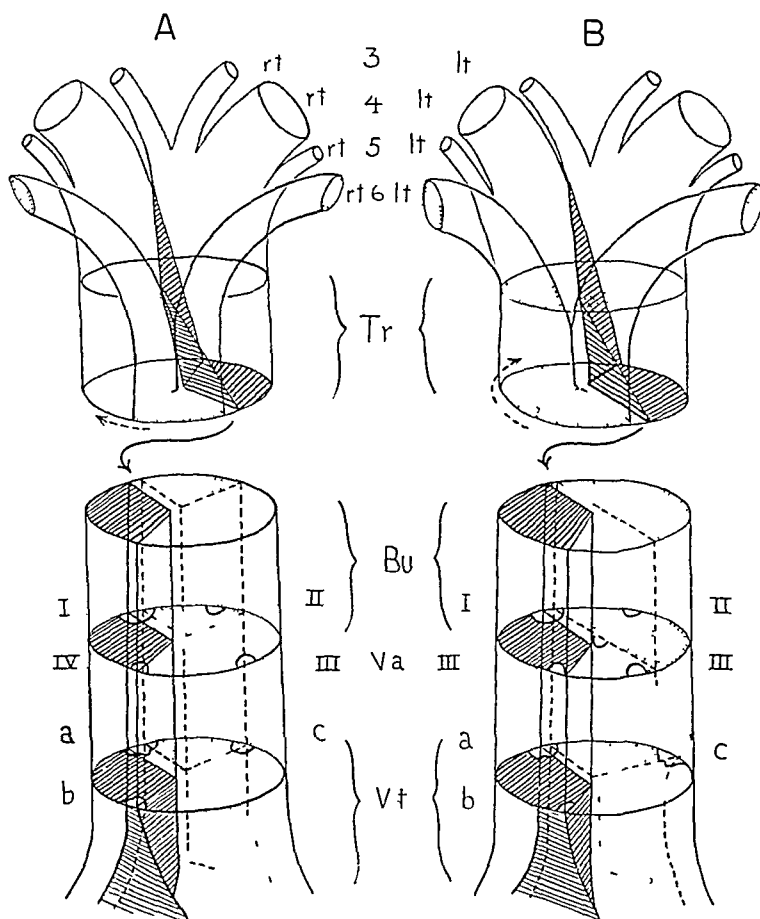


Fig 4—Diagrammatic representation of the truncus bulbus region according to the theory of Spitzer The truncus is pictured as a straight tube The upper portions are rotated about the lower segments because of the clockwise spiral of the truncus—denoted by the heavy arrow The stage in cardiac development is just prior to the division of the truncus into the great vessels The heart is viewed from above and posteriorly In the cross sections the anterior portions are above and the posterior portions are below

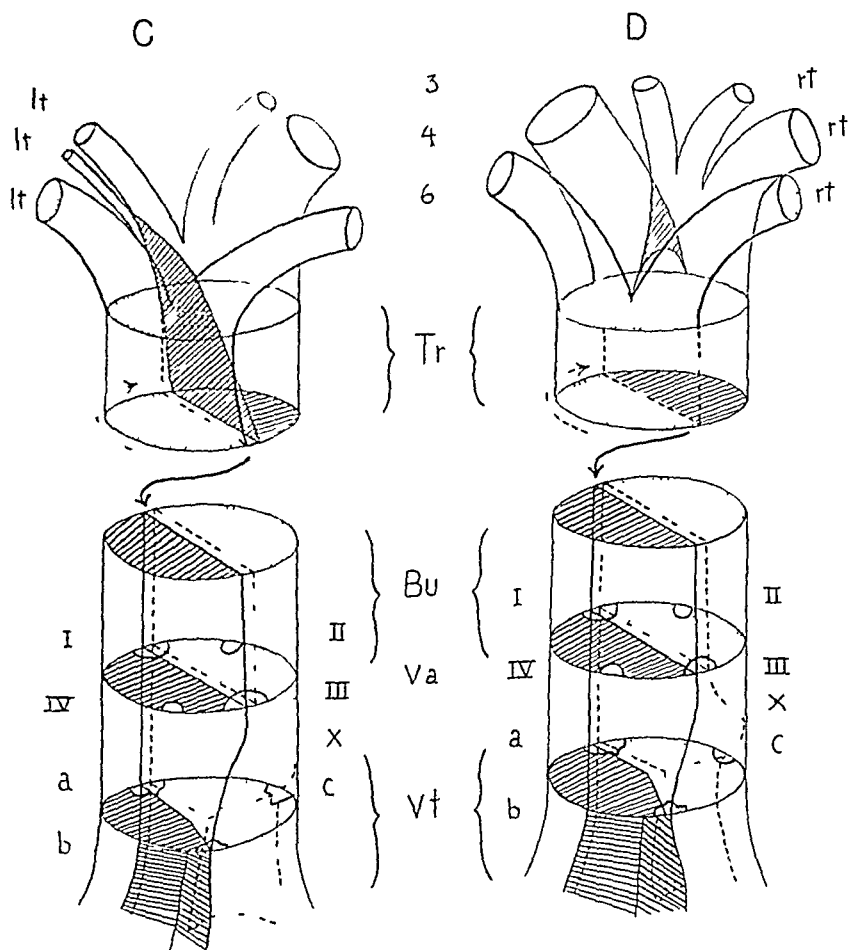
*A*, shows the original form, *B*, the reptilian form, *C*, the avian form, and *D*, the mammalian form

*Tr* indicates the level of the truncus, *Bu*, the level of the bulbus, *Vt*, the level of the ventricular base, and *Va*, the level of the great vessel valves

The heavily stippled area is the pulmonary artery, the lightly stippled area, the crista supraventricularis The line shaded area at the level of the truncus

distal bulbar swellings I and III have the entire task of separating the two blood streams, these will dominate in ontogeny

*Mammalian Form* (fig 4 D) —The force of the pulmonary circulation is felt very early in mammalian development. The flow from the left ventricle increases so rapidly that both fourth arterial arches must remain open for a time to take care of it. The development of the septum aorticum in the truncus region is thus delayed while the develop-



is the septum aorticum, that at the level of *Vt*, the interventricular septum, that in each cross section, the left ventricular aorta. The unshaded areas in the cross sections represent the right ventricular aorta. In the cross sections the broken lines indicate the septum aorticopulmonale, the straight lines, the septum aorticum.

3 *lt* indicates the left third aortic arch, 3 *rt*, the right third aortic arch, 4 *lt*, the left fourth aortic arch (obliterated in C), 4 *rt*, the right fourth aortic arch, 5 *lt*, the left fifth aortic arch (obliterated in C and D), 5 *rt*, the right fifth aortic arch (obliterated in C and D), 6 *lt*, the left sixth aortic arch, and 6 *rt*, the right sixth aortic arch.

I, II, III, IV indicate the distal bulbar swellings, a, b, c, the proximal bulbar swellings, x, the niche of the obliterated right ventricular aorta.

The broken arrow shows the gradual enlargement of the pulmonary artery through phylogeny.

ment of the septum aorticipulmonale separating the pulmonary artery from the aorta is hastened. For the same reason, in the ventricular region the development of the septum aorticum, which separates the arterial from the venous blood, is rapid. Human embryos have a composite or secondary septum aorticipulmonale constructed of the septum aorticum below and the septum aorticipulmonale above. These fuse in the midportion of the bulbus. The blood may now take either the left or the right fourth arterial arch, utilizing the left because it is thrown in that direction by the clockwise spiral of the truncus.

In the ventricular region proximal swellings *a* and *b* form the septum ventriculorum, while swelling *c* is situated in the right ventricle. As in the birds, the latter is small. From the foregoing theory, however, one would expect to find it posterior to the pulmonary artery and anterior to the tricuspid valve, enclosing between it and the valve the remnants of the right-chambered aorta. Human hearts show the crista supraventricularis and the trabecula septomarginalis (or moderator band) in this position with a small niche between the crista and the anterior tricuspid leaflet. This niche, according to Spitzer, represents the remnant of the right ventricular aorta.

The theory also explains why each of the three great vessels of reptiles possesses two valve cusps, while each of the two vessels of birds and mammals has three. In the latter types the valvular portion of bulbar swelling III is shared by both vessels, as it is split by the migration of the posterior leaf of the septum aorticum.

*Bulboatrial Ledge* (fig 5).—There remains for consideration Spitzer's hypothesis concerning the fate of the bulboatrial ledge, which originally jutted in between the descending and ascending limbs of the ventricular loop. In this position it separated the incoming and outgoing streams of the ventricles, and its remnants must be in similar relations in the adult, between the atrioventricular valves and the great vessels. With the growth of the left aorta, the left leaf of the bulboatrial ledge is pushed down and back, while the obliteration of the right-chambered aorta causes the right leaf to move forward and upward. This results in a partial left spiral and places the ledge in a position to complete the right spiraled edge of the interventricular foramen. Its course is pictured in figure 5, and it will be noted that this can explain why the septum membranaceum separates the left ventricle from both the right atrium and the right ventricle. The left leaf fuses with the aortic leaflet of the mitral valve, while the right fuses with the large anterior ledge of the tricuspid valve.

Spitzer brought forth a number of points in proof of his theory

- 1 The niche between the crista supraventricularis and the anterior tricuspid leaflet shows the position of the former right-chambered aorta

2 Tandler's illustration of the early human embryo clearly shows the disposition of the three proximal swellings in the ventricular region<sup>11</sup>

3 Tandler recognizes the homology of the *Muskelleiste* of the reptile with the trabecula septomarginalis of man

4 Geil states that the growth of the partitions between the aortic arches is the essential bridge for the union of the bulbar swellings

5 Langer's illustrations show the movements of the septal portions of bulbar swelling II to bulbar swelling III in embryos of *Lacerta*

6 The migration of the septal portions of the distal bulbar swellings of bird embryos is, as pictured by Langer, in accord with the theory

7 Langer made the "strange finding" that the septal portion of distal bulbar swelling I in reptiles continues as part of IV but in human embryos wanders over the distal bulbar swelling III

8 Evidence has been adduced from human malformations

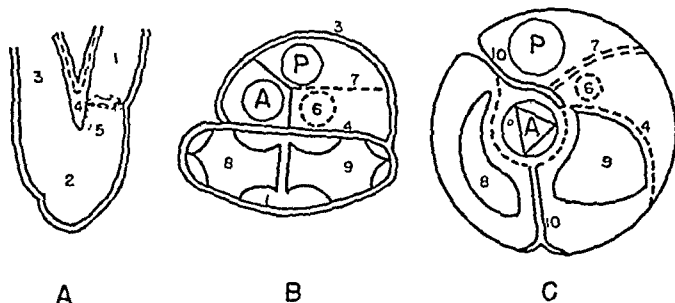


Fig 5—Diagrams illustrating Spitzer's views concerning the fate of the bulbo-atrial ledge (after Brings and Spitzer, 1928) *A* represents a sagittal section of the primitive cardiac loop, *B*, a cross section of the ventricular loop as viewed from above (the auricular structures having been projected onto the same plane), and *C*, the same as *B* but at a later stage in development. Within these diagrams *A* is the aorta, *P*, the pulmonary artery, *1*, the auricular portion of the cardiac loop, *2*, the ventricular portion of the cardiac loop, *3*, the bulbar portion of the cardiac loop, *4*, the bulboatrial ledge, *5*, the atrioventricular valve, *6*, the site of the obliterated right ventricular aorta, *7*, the crista supraventricularis, *8*, the mitral valve, *9*, the tricuspid valve, and *10*, the interventricular septum

#### APPLICATION OF THE PHYLOGENETIC THEORY OF SPITZER TO CARDIAC MALFORMATIONS

Spitzer's theory of normal cardiac development delegates to the torsion of the heart loop as determined by changes in pulmonary circulation a function of paramount importance in the formation of the septums. It is therefore understandable that he should have ascribed cardiac anomalies to inadequacy of this stage. Thus, decrease in the amount of torsion may lead to incomplete formation of the septums. This concept differs widely from that of Rokitsansky, of Geipel and of Pern-

<sup>11</sup> See figure 25, page 34, in Tandler's "Anatomie des Herzens"

kopf and Wirtinger inasmuch as it refers to detorsion of the entire heart relative to the normal position and not to insufficient spiraling of an isolated segment. Furthermore, the insufficient torsion is but the first step of a series of events which lead to the malformation. The heart is placed in an earlier phylogenetic position where torsion had not taken place to such a great extent. Then the forces of the blood stream, still working in ontogeny, can bring development to a phylogenetically and mechanically determined conclusion. The result will differ from the normal. Spitzer inferred that there is no complete departure from developmental principles, as is postulated by other theories in the deficient spiraling of the truncus septum and the consequent union of nonhomologous structures. These principles have been too firmly grounded in phylogeny since the onset of lung breathing in the fishes to allow so radical a change. The inevitable corollary to these statements is that complete homology must be present between all structures in malformations and those in the normal heart. Transposition must be apparent and not real. In any stage of phylogenetic development the septum aorticum separates the right from the left aorta, while the septum aortico-pulmonale and its continuation (the crista supraventricularis in the mammal) separate the pulmonary artery from the aortas. These relations cannot change because ontogenetic factors happen to complete an earlier stage in phylogenetic development.

These basic principles are the guide to the various types of transposition as set forth and graded by Spitzer according to the probable degree of detorsion which had taken place.

*Type 1 Overriding Aorta* (fig. 6) — Slight detorsion (or, actually, a smaller than normal degree of torsion) has taken place. Septum development is hindered, and those portions farthest removed from the forces of the blood streams cannot develop completely. The already proximally withdrawn septum aorticum and its immediate ventricular prolongations are first involved and disappear. This results in an upper interventricular defect and fusion of the two aortas (implying an opening of the right-chambered aorta). The detorsion also causes the bulbus cordis to be rotated in a counterclockwise direction, placing the common aorta more to the right, the pulmonary artery more to the left, and the crista more anterior and at a more acute angle to the septum ventriculorum. In *ontogeny* the outflow through the right-chambered aorta is increased, while that through the pulmonary artery is decreased. Moreover, the crista, now dividing the two main streams, is subject to the same forces which normally cause the development of the septum ventriculorum. Its potentialities as a septum become evident, and it hypertrophies. The pulmonary artery becomes smaller as a consequence of the smaller volume of blood and because it is compressed between the hypertrophied crista and the septum ventriculorum. Phylogenetically

speaking, the detorsion prevents the complete migration of distal bulbar swelling II to III. The valvular portion of swelling III is not split and divided between the aorta and the pulmonary artery. Both from ontogenetic and phylogenetic factors, therefore, a stenotic and also bicuspid condition of the pulmonary artery arises if the detorsion is of the proper degree.

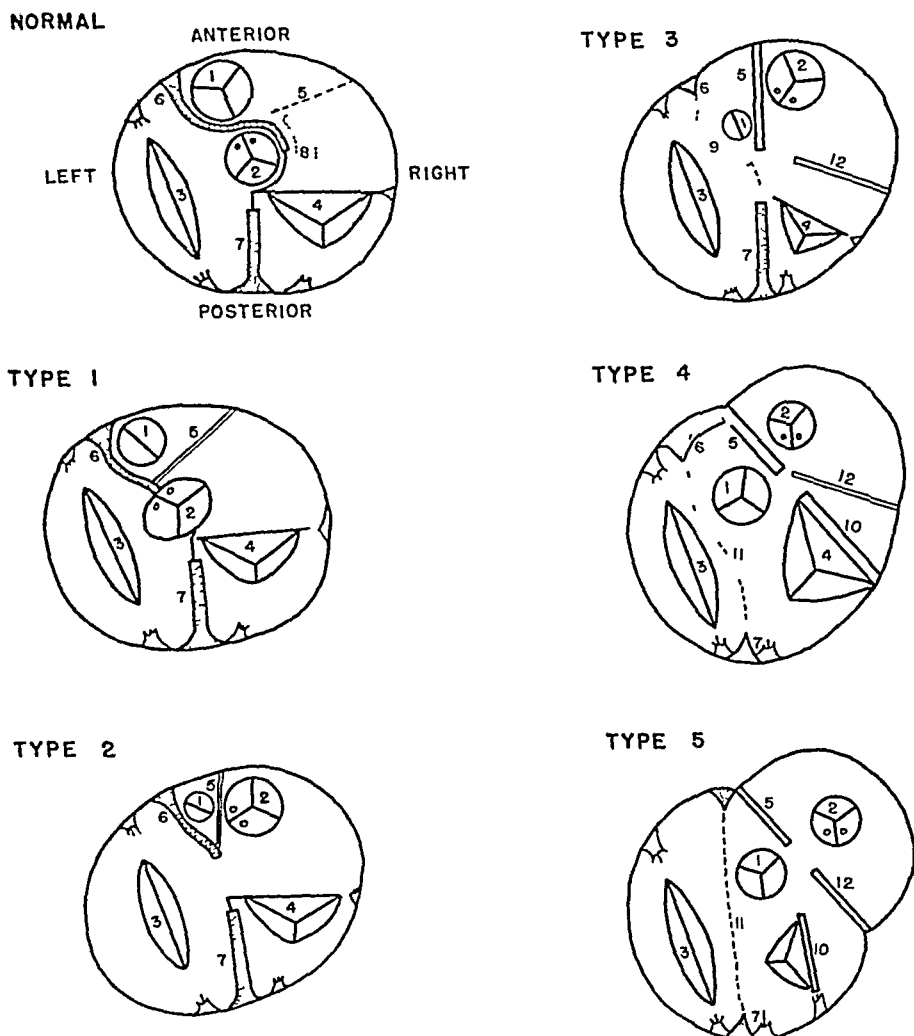


Fig 6—Diagrams to show the various structures of the heart in the normal position and in the different types of transposition (after Spitzer, 1923 [c]). The structures are projected on the ventricular base, which is viewed from above and posteriorly. 1 indicates the pulmonary artery, 2, the aorta, 3, the mitral valve, 4, the tricuspid valve, 5, the crista supraventricularis, 6, the anterior portion of the interventricular septum, 7, the posterior portion of the interventricular septum, 8, the site of the obliterated right ventricular aorta, 9, the site of the obliterated left ventricular aorta, 10, the anterior tricuspid ledge, 11, the site of the undeveloped true interventricular septum, and 12, the bulboatrial ledge.

The detorsion is seen also in deficient spiraling of the aorta about the pulmonary artery and in the relative clockwise torsion of the aorta and pulmonary artery, as determined by the position of the cusps. The

coronary-bearing sinuses thus appear in left anterior and left posterior position instead of right anterior and left anterior position

In summary, the characteristics of this type would be an overriding aorta, a hypertrophied crista supraventricularis, an interventricular defect, a stenotic and bicuspid pulmonary valve, a diminished spiraling of the pulmonary artery about the aorta and a counterclockwise rotation of the coronary cusps. These characteristics are illustrated by cases 1 through 5 of the present series (page 456). An increasing grade of detorsion is shown by the fact that in case 1 the aorta is situated mainly over the left ventricle and in case 5 mainly over the right ventricle. The pulmonary artery is stenotic, being completely atretic in case 5, in which the crista supraventricularis has probably fused with the anterior septum ventriculorum, thus obliterating the pulmonary conus.

*Type 2 Simple Transposition of the Aorta*—An increase in the amount of detorsion has taken place, as shown by the fact that the pulmonary artery is farther to the left. The aorta is placed more to the right, the crista is directed more sagittally and the coronary arteries appear more counterclockwise in position than in the normal heart. The detorsion removes the left aorta from its connection with the left ventricle. The flow through it ceases, and it is obliterated. In contrast, the right aorta is placed directly above the right ventricle and reopens. In this manner both pulmonary artery and aorta arise from the right ventricle, and an apparent transposition of the aorta has taken place. Actually, however, the left aorta has closed and the right reopened. *No true transposition has occurred.* The pulmonary valve is still bicuspid and stenotic, while the crista is more hypertrophied than in type 1. In some cases this hypertrophy has been so great that the crista appears to be an accessory septum. As an example there may be cited a case of Rokitsky's (3196) in which a false anterior septum enclosed, with the true anterior septum, a third ventricle giving rise to the pulmonary artery. This condition is illustrated by case 2 of this series. No examples of uncomplicated type 2 transposition are present in the collection at the Children's Hospital. Cases 7, 7a and 7b show mitral atresia and a rudimentary state of the left ventricle in addition to a type 2 transposition.

*Type 3 Crossed Transposition*—In this group evidences of greater detorsion are present. The aorta and pulmonary artery issue from the base in the position indicated in figure 6 and pursue a more or less parallel course upward. As in the preceding group the right aorta has opened, while the left has closed. Because of the greater detorsion, this occurs earlier, and the ventricular prolongation of swelling *a* is so far removed from the two emergent streams that it cannot develop to a

septum Swelling *c* is now directly between the aortic and pulmonary streams and in consequence undergoes hypertrophy If, because of the detorsion swelling *c* reaches a position directly in line with the posterior swelling *b* (in other words, occupies a position comparable to the normal position of *a*) it may develop as *a* does normally and form a false anterior septum This throws the pulmonary artery into what is apparently a left ventricle, while the right-chambered aorta remains in the right ventricle A crossed transposition apparently takes place If, on the other hand, the crista is not in line with the posterior portion of the septum ventriculorum, neither can develop to a sufficient extent and the septum remains rudimentary A cor biloculare or triloculare univentriculorum with the aorta to the right of the pulmonary artery, results Since, in this group, the pulmonary artery receives the full current from the left ventricle, and since it is not hemmed in between the crista and the anterior true septum, it undergoes enlargement and regains the tricuspid condition

If one examines the position of the bulboatrial ledge, it will be noticed that anterior movement of the right-chambered aorta will tend to draw the right leaf of the ledge anteriorly, separating it from the anterior ledge of the tricuspid to which it had been fused In the new position the ledge may undergo hypertrophy to form a band between the aorta and the anterior tricuspid ledge It is therefore in the position of, and may appear to be, the crista supraventricularis This, argued Spitzer, cannot actually be true, since the crista must always lie between the pulmonary artery and the right-chambered aorta (in situs solitus)

Case 6 is an example of a transitional form between types 1 and 3 The aorta is almost entirely over the right ventricle, while the pulmonary artery, though at first directed toward the right, finally ends in a left ventricle Cases 8 through 11 demonstrate type 3 transposition Case 16 shows the combination of type 3 transposition and bulboventricular inversion

*Type 4 Mixed Transposition*—With increasing grades of detorsion, the crista may come into line with the posterior tricuspid ledge, the anterior tricuspid ledge or the right leaf of the bulboatrial ledge Should a false septum develop in the second case, the tricuspid valve will apparently enter the left ventricle (type 4) The large anterior cusp of the valve then spans the interventricular septal defect, since the anterior tricuspid ledge forms part of the septum Were a false septum to develop from the crista supraventricularis and the bulboatrial ledge the tricuspid valve would be transposed entirely into the left ventricle (we shall call this condition type 5) (see page 486) In both type 4 and type 5 the right ventricle becomes a pure aortic chamber Stenosis of the aorta results because of insufficient blood flow If the direction of the crista lingers long between the planes of the anterior and posterior



tricuspid ledges, both the latter may hypertrophy, encroaching on and obliterating the tricuspid valve between them

Case 12 is an example of a transitional form between types 2 and 4. Two incomplete ventricular septums are present. The first divides the heart so as to place the aorta into a right ventricle (type 2), while the second demarcates a pure aortic ventricle. Case 13 demonstrates transposition of the tricuspid valve, while cases 14 and 15 show the aortic ventricle. In case 17 the transposition is associated with obliteration of the tricuspid valve. In addition there is bulboventricular inversion.

*Transposition of the Coronary Arteries*—As the detorsion takes place and the aorta winds around the pulmonary artery in a counterclockwise direction, the right coronary stem will successively cross branches of the left. As it does, these branches may acquire secondary union with the right stem and lose their original connection with the left. In a similar fashion the branches of the right coronary artery may be transposed to the left coronary. The coronary arteries have been examined in all the cases of this series. It will be seen that an increasing degree of coronary transposition occurs from type 1 to type 4.

#### PRESENTATION OF CASES TYPE 1 OVERRIDING AORTA

CASE 1—*Hypertrophy and rotation of entire heart, closed foramen ovale, interventricular septal defect, patent ductus arteriosus, hypertrophied crista supraventricularis, right ventricular preponderance, overriding aorta, stenosis of pulmonary artery, bicuspid pulmonary valve, rotation of orifices of coronary arteries,<sup>12</sup> persistent right aortic arch, complete vascular circle around trachea and esophagus*

A 4 day old girl showed numerous external congenital malformations, irregular and feeble respirations, subnormal temperature, cardiac enlargement and cyanosis of varying degree from the time of birth. No cardiac murmurs or thrills were noted. At autopsy the following additional observations were made: hemorrhagic bronchopneumonia, bilateral harelip, cleft palate, horseshoe kidney with hypoplasia of the right half and atresia of the right ureter, clubfeet, incomplete rotation of the cecum, deformity of the right external ear and hypoplasia of the labia majora vulvae. The microscopic structure of the heart was found not remarkable.

The heart is greatly enlarged and is ovoid. The long axis, through the apex, is directed to the left and slightly downward, so that the heart tends to lie in a transverse direction. The transverse diameter is 6 cm. in contrast to the trans-thoracic diameter of 9 cm. When viewed anteriorly, the pulmonary artery is seen to arise from the center of the cardiac base. The aorta arises to the right between the pulmonary artery and the markedly enlarged right auricle, which projects forward around the right side of the heart. The left atrium cannot be seen anteriorly. The anterior interventricular sulcus courses very close to the left margin and ends in a small groove to the left of the apex. The posterior sulcus is more obliquely placed, running downward and to the left. The right ventricle is approximately twice as large as the left.

<sup>12</sup> That is, rotation of the aorta, thus carrying the coronary-bearing aortic sinuses into the more counterclockwise position with reference to the normal

The right atrium receives the superior and inferior venae cavae in a normal manner. Just below the prominent eustachian valve is the orifice of the coronary sinus, guarded posteriorly by a rudimentary valve. The fossa ovalis measures 1.5 cm in diameter and is completely closed by a thin membrane which bulges into the left atrium. The right atrioventricular valve, measuring 45 mm in circumference, has three cusps, anterolateral, medial and posterior. The cusps show a row of irregular transparent nubbins along the free margins. The remainder of the leaflets is not remarkable. The anterior papillary muscle is relatively small and is inserted into the anterolateral wall of the ventricle on a ridge which continues medially to reach the interventricular septum at the commencement of the crista supraventricularis. This ridge is the moderator band (*trabecula septo-marginalis*). The moderately hypertrophied crista supraventricularis arches superiorly, laterally and anteriorly across the ventricular base, from the interventricular septum to the anterolateral ventricular wall near the anterior papillary muscle. It encloses a small space between it and the anterior portion of the interventricular septum. This space is the lower orifice of the pulmonary conus. To the posterior aspect of the crista are attached several chordae tendineae which run to the anterior cusp of the tricuspid valve. Between the crista supraventricularis and the medial cusp of the tricuspid valve is a deep recess which leads at its apex to a defect in the interventricular septum. The pulmonary artery is narrowed at its origin, measuring 22 mm in circumference. A well formed bicuspid pulmonary valve is present. The cusps are situated in left anterior and in right posterior position when viewed in situ. Immediately distal to the valve there is a slight dilatation of the pulmonary artery.

The left atrium is much smaller than the right atrium. It receives two pulmonary veins at its right extremity and one at its left. The latter divides shortly into two branches. The membrane covering the fossa ovalis bulges into the atrium. The auricular appendage is small and narrow. The mitral valve and papillary muscles do not show any abnormality. The interventricular septum is thick in its inferior portion but becomes narrower above to present a semicircular defect in its upper posterior portion. The membranous septum is not present. Above the defect is the base of the aorta, which is situated more over the left ventricle than over the right. The aortic valve is tricuspid, with its leaflets placed in right posterior, anterior and left posterior position. To the right side of the aortic base is attached part of the medial leaflet of the tricuspid valve, which thus obscures, with the anterior tricuspid leaflet, the opening of the interventricular defect into the right ventricle. The left posterior margin of the aortic base continues down to the aortic cusp of the mitral valve.

The coronary arteries arise from the sinuses behind the anterior and left posterior aortic cusps. The former corresponds to the right coronary artery and courses around the right atrium to terminate in the posterior interventricular branch. The latter gives off the anterior interventricular artery and the left circumflex branch.

The further course of the great vessels is of interest. The aorta gives rise to the left common carotid artery, which passes up anterior to the pulmonary artery. The aorta then passes superiorly and posteriorly to reach the right side of the trachea. Opposite the trachea the aorta gives rise anteriorly to the right common carotid artery and then passes behind the esophagus. The right and left subclavian arteries arise from this portion of the aorta. The main pulmonary artery courses from the cardiac base to the left of the trachea, where it branches. The right pulmonary artery runs between the aorta and the trachea. The left pul-

monary artery passes directly to the left lung. At the bifurcation of the pulmonary artery there is a large patent ductus arteriosus, which joins the aorta after the latter has reached the left side of the esophagus. A complete vascular ring is thus formed around the esophagus and trachea.

*CASE 2 (fig 7) — Hypertrophy and rotation of entire heart, closed foramen ovale, interventricular septal defect, closed ductus arteriosus, accessory septum in right ventricle, right ventricular preponderance, overriding aorta, rudimentary and stenotic pulmonary valve*

A 10 week old boy was well until the sixth week of age, when he began to have spells of choking and coughing. Severe dyspnea and convulsions occurred shortly before his entry into the hospital. Physical examination revealed expiratory stridor, dyspnea, marked cyanosis, a harsh systolic murmur over the precordium, best heard in the tricuspid area, a soft middiastolic murmur in the same area, dulness and rales at the bases of the lungs, and mild hepatomegaly. Death occurred several hours after admission. At autopsy pulmonary congestion was observed. The microscopic structure of the heart was found not remarkable.

The heart weighs 50 Gm (normal weight, 23 Gm)<sup>13</sup>. It lies transversely in the thoracic cavity, with a vertical diameter of 4.5 cm and a transverse diameter of 7 cm. In addition, it is apparently rotated in a 90 degree arc about its long axis so that the anterior interventricular groove is at the left border and the posterior interventricular groove courses down the right border. The anterior surface of the heart is thus comprised entirely of the right ventricle. The septum ventriculorum is in a frontal plane. This brings the right atrium in front and a little to the left of the left atrium, a part of which is visible at the extreme right border of the heart. Both great vessels arise from the posterior portion of the cardiac base, the aorta anterior and to the right of the pulmonary artery. The right ventricle has a large rounded anterior projection at its upper left portion. It measures 3 by 3 cm in diameter and extends forward 2 cm. The pulmonary artery arises from the summit of this projection.

For easier comprehension, the heart will be described as though it had the normal position in the thoracic cavity with the anterior interventricular groove coursing near the left border and the septum ventriculorum placed in a sagittal plane. This allows the atria and ventricles to be described as right and left as usual instead of anterior and posterior as is actually the case.

The right atrium is not remarkable. The foramen ovale is completely closed. The veins and their valves are in their normal position. The right atrioventricular valve measures 30 mm in circumference and contains anterior, medial and posterior cusps. The anterior papillary muscle is very large and is inserted into the right ventricular wall close to the lower anterior portion of the interventricular septum. The posterior muscle is small. The large medial muscle of Lancisi takes origin from the interventricular septum. The three papillary muscles<sup>\*</sup> are in close approximation to one another and thus reduce the posterior, or inflow, portion of the right ventricle to a small chamber. The remainder, or outflow portion, of the right ventricle is heavily trabeculated and is divided into a larger anterior portion and a smaller posterior portion by a false septum which corresponds in position to the crista supraventricularis (fig 7). It extends downward at right angles to the true interventricular septum. The false septum is 1.5 cm deep and leaves but a small oval opening inferiorly, measuring 5 mm in diameter, between the two portions of the anterior part of the right ventricle. Behind the false

<sup>13</sup> The normal weights are taken from Coppoletta and Wolbach.

septum and between it and the anterior leaflet of the tricuspid valve is a deep niche which leads up to the aorta and a defect in the true septum. In front of the false septum is an accessory chamber, measuring approximately 2 by 2 cm, which forms the projection noted on the surface of the right ventricle. The chamber leads at its summit to the pulmonary artery. The pulmonary valve measures 18 mm in circumference and is represented by a thickened irregular band of tissue rather than by definite cusps. The artery gives off its two branches in the usual manner. A ductus arteriosus cannot be found. The left atrium, bicuspid valve (measuring 38 mm in circumference) and papillary muscles are not remarkable. The interventricular septum is thick and intact save for an oval-shaped defect, 7 by 3 mm, in the upper anterior portion. This defect is surmounted by the aorta, which opens, for the most part, into the left ventricle. The interventricular defect connects the outflow portion of the left ventricle with the small

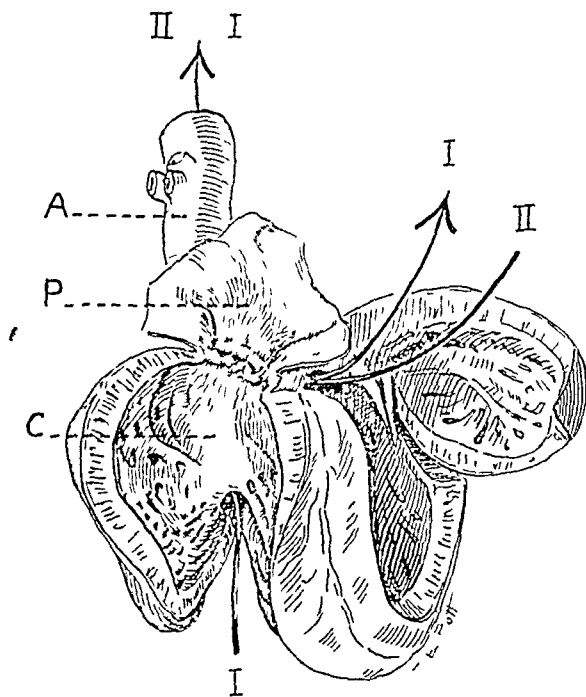


Fig 7 (case 2)—View of the heart from the left. Both ventricles have been opened. The interventricular septum lies vertically to the plane of the paper. Its position is shown by the anterior descending coronary artery. *A* indicates the aorta, *P*, the pulmonary artery, and *C*, the markedly hypertrophied crista supraventricularis separating a small pulmonic ventricle from the remainder of the right ventricle. Arrow *I* passes through the right ventricle into the aorta and through the septal defect into the left ventricle. Arrow *II* passes from the left ventricle into the aorta.

middle, or aortic, chamber of the right ventricle. The aortic valve has three cusps, which lie in right, left and posterior position. The right and left sinuses give off the usual coronary arteries. The aortic valve measures 30 mm in circumference. The further course of the great vessels is not abnormal. The left ventricular muscle measures 5 mm and the right 4 mm in thickness.

*CASE 3—Persistent ostium primum, common atrioventricular orifice, interventricular septal defect, patent ductus arteriosus, overriding aorta, normal pulmonary artery, hypertrophy of right ventricle*

A 2 day old boy showed cyanosis and feeble respirations from the time of birth, with rapid development of persistent abdominal distention, vomiting and bleeding from mucous membranes. The heart was not enlarged nor were murmurs heard. Roentgen examination showed a normal heart shadow. At autopsy the following conditions were observed: hemorrhagic necrotizing gastroenterocolitis, hemothorax, hemopericardium, congenital anomalies of mesentery and duodenum but no intestinal atresia. Microscopic examination of the heart showed only congestion and small hemorrhages.

The heart is not enlarged and is normal in shape. The apex is directed downward and to the left. The interventricular grooves are normal in position. The coronary arteries are unusually prominent.

The interatrial septum is defective. It is present to a slight extent on the superior and posterior walls of the atria. The fossa ovalis and eustachian valve are not present. The defect in the atrial septum measures 1 cm in diameter and merges below with a large defect (15 by 6 mm) in the upper portion of the interventricular septum. The right atrium receives the venae cavae and coronary vein while the left receives the pulmonary veins in normal position.

There is only one atrioventricular valve because of the fusion of the posterior cusp of the tricuspid with the posterior half of the anterior leaflet of the mitral valve and the fusion of the anterior half of the latter cusp with the medial cusp of the tricuspid valve. The right ventricle is slightly larger than the left. At the left anterior portion of the right ventricle a rather small infundibulum leads into the pulmonary artery. The pulmonary valve is tricuspid and measures 22 mm in circumference. Behind the infundibulum a hypertrophied crista supraventricularis arches upward, laterally and slightly forward from the upper third of the interventricular septum to the right wall of the ventricle. It gives rise to several chordae to the anterior and medial cusps of the tricuspid valve. Between the crista and the anterior tricuspid leaflet is a niche which leads to the anterior portion of the interventricular septal defect and to the aortic valve. The left ventricle presents a mitral valve which has three cusps because of the splitting of the aortic leaflet. Arising from the middle of the cardiac base, between the anterior half of the anterior mitral leaflet on the left and the medial and anterior leaflets of the tricuspid valve on the right, is the aorta, which overrides the anterior portion of the large interventricular defect. It measures 15 mm in circumference and drains both ventricles. The aortic valve is tricuspid, containing anterior left, anterior right and posterior leaflets. The sinuses above the two anteriorly placed cusps give rise to normal right and left coronary arteries. The aorta decreases slightly in caliber until it joins the patent ductus arteriosus, at which point it becomes wider.

*CASE 4—Hypertrophy and detorsion of heart, functionally closed foramen ovale, interventricular septal defect, patent ductus arteriosus, right ventricular preponderance, clockwise rotation of crista supraventricularis, overriding aorta, marked stenosis of pulmonary artery, bicuspid pulmonary valve, rotation of coronary artery orifices*

A 4 month old girl was well up to seventeen days before death, when she began to show irritability, fever, convulsions and signs of a cerebral disturbance on the right. Roentgenograms showed slight cardiac enlargement. A soft systolic

murmur was heard over the precordium. At autopsy the infant showed, in addition, bronchopneumonia, a single lobe of the left lung being involved. The cranial vault was not examined.

The right ventricle of the heart is prominent, making up almost the entire anterior surface and approximately one half of the posterior surface of the heart. The pulmonary conus is well marked exteriorly and gives rise to a very narrow pulmonary artery. The large aorta arises posteriorly and to the right of the pulmonary artery. The pulmonary artery arises from the extreme left upper portion of the heart. The anterior descending coronary artery is unusually prominent as it courses down the ventricle near the left cardiac border.

The right atrium is large, measuring 20 by 15 mm. The openings of the veins and coronary sinus are normally situated. The eustachian valve is prominent, and the thebesian valve is fenestrated. The fossa ovalis measures 9 mm in diameter and has a small slitlike aperture anteriorly, which was probably closed during life. The tricuspid valve measures 40 mm in circumference and bears three leaflets in normal arrangement. The papillary muscles are of moderate size and are situated in their usual positions. The architecture of the anterior, or outflow, portion of the right ventricle is complex. The ventricular septum shows a defect anteriorly and superiorly, extending from the atrioventricular region to the anterior wall and measuring 10 by 4 mm. Directly above this defect is the aorta, which is shared equally by both ventricles. The base of the aorta is bounded on the right side by the medial tricuspid leaflet and the small papillary muscle of Lancisi. The right anterolateral border of the aortic base is defined by a hypertrophied ledge which arises from the upper portion of the interventricular septum anteriorly, arches over the ventricular base just in front of the aorta and then runs to the anterolateral ventricular wall to become lost between the anterior and posterior papillary muscles of the tricuspid valve. The medial portion of this ledge is closely applied to the anterior ventricular wall, leaving but a small slitlike aperture, which leads to the pulmonary artery. The ledge, therefore, corresponds in position to the normal crista supraventricularis, which, by assuming a more anterior course, has almost obliterated the pulmonary conus. The pulmonary valve is very narrow, measuring 10 mm in circumference. It contains two thickened cusps, which are situated right anteriorly and left posteriorly. The opening between them is a mere slit. Distal to the valve, the artery becomes wider and gives off the pulmonary branches, which have the usual course. The ductus arteriosus is 1 cm long and admits a 2 mm probe.

The left atrium is not remarkable save for its small size. It measures 20 by 5 mm. The openings of the pulmonary veins, the mitral valve (measuring 29 mm in circumference) and its papillary muscles are not remarkable. The anterior portion of the interventricular septum shows the rounded defect which is overridden by the aorta. The aortic valve measures 35 mm in circumference and bears right posterior, anterior and left posterior cusps. In the sinuses behind the latter two cusps are the orifices of the coronary arteries. The left posterior cusp has the usual close relation to the aortic leaflet of the mitral valve. The right posterior cusp seems to run directly into the medial cusp of the tricuspid valve. From the junction of the right posterior and anterior cusps of the aorta, a large muscle band, measuring 4 mm in width and 15 mm in length, runs down to the right and forward to pass behind the crista supraventricularis, which is applied to the anterior ventricular wall at this point. The band is inserted in front of and to the left of the anterior papillary muscle of the tricuspid valve. The right coronary artery takes origin from the anterior sinus, while the left arises from the left

posterior sinus. The branches are normal. The branches of the aorta are not remarkable. The left ventricular myocardium measures 4 to 5 mm in thickness, while the right measures 5 mm.

*CASE 5—Hypertrophy and detorsion of heart, open foramen ovale, interventricular septal defect, apparent absence of crista supraventricularis, right ventricular preponderance, overriding aorta, atresia of pulmonary orifice, rudimentary pulmonary artery, counterclockwise rotation of coronary-bearing aortic sinuses, angulation of planes of atrial and ventricular septums, patent ductus arteriosus*

A 6 month old boy showed cyanosis at birth and on exertion thereafter. There were daily spells of respiratory difficulty. Cyanosis and crying preceded bowel movements. A faint mitral systolic murmur was heard. Death followed shortly after an acute nutritional disturbance. Examination before death showed dyspnea, cyanosis, tachycardia and cardiac enlargement. A systolic murmur was heard all over the precordium, most marked over the sternum. The pulmonic second sound was diminished. There were no thrills or diastolic murmurs. Additional observations at autopsy were tracheobronchitis, cerebral congestion and acute gastritis. The histologic examination of the heart showed nothing remarkable.

The heart is very much enlarged. It lies almost transversely, with the apex pointing to the left and slightly downward. The anterior interventricular groove is situated far to the left, running sagittally down toward the apex. The entire heart is turned so that the ventricular septum is placed at a 45 degree angle between the sagittal and frontal planes. The interatrial septum is in the sagittal plane, at an acute angle to the ventricular septum. The obtuse margin of the heart is very short while the acute margin is long and forms the right and inferior borders of the organ. At first only one large vessel is seen to arise from the ventricles. This comes off slightly to the left of the midline and courses almost directly upward. After dissection a small thin vessel is found attached to the left posterior side of the larger vessel. It runs down around the left side and in front of the larger vessel to be inserted in the right ventricle just to the right of the interventricular groove. The larger vessel is the aorta and the smaller is the pulmonary artery. The right atrium is large and projects around the lateral side of the aorta. Only a small portion of the left auricular appendage is seen anteriorly. The entire right side of the heart is much hypertrophied and seems to give rise to both vessels.

The right atrium measures 35 mm in the transverse and 20 mm in the vertical diameter. The superior and inferior venae cavae and the coronary sinus arise in normal positions. The eustachian valve is intact, but the thebesian valve is merely a network of fine fibers on the atrial wall posterior to the coronary opening. The limbus fossa ovalis is prominent. The fossa ovalis measures 3 cm in circumference. The posterior portion of the fossa is closed by a membrane which has a free crescentic anterior border. Between this and the prominent anterior margin of the limbus is an oval defect measuring 5 by 4 mm. The right atrioventricular orifice measures 40 mm in circumference and has three cusps in essentially normal positions. The papillary muscles are small. The anterior muscle is inserted in a mass of trabeculae in the right lateral portion of the ventricle. In the anterosuperior portion of the septum in front of the atrioventricular orifices is an oval defect, which is surmounted by the aorta. No remnants of a crista supraventricularis, moderator band or conus arteriosus in the right ventricle can be identified. The ventricle is heavily trabeculated throughout. The interventricular septal defect measures 13 by 4 mm and is situated beneath the left

side of the aorta. The base of the aorta is bounded by the right anterolateral ventricular wall, the anterior cusp of the tricuspid laterally, the medial cusp laterally and posteriorly, and the mitral valve on the left side. Three quarters of the aortic base lies over the right ventricle. The aortic valve measures 35 mm in circumference and bears three normal cusps, situated in right lateral, anterior and left posterior position. The aorta courses upward and to the left, giving off the innominate, left common carotid and left subclavian arteries in that order. At the apex of the arch the aorta gives off a small vessel measuring 1 mm in diameter, which courses down its left side. This vessel gives off the left pulmonary artery, then the larger right pulmonary artery, which runs behind the aorta. The original vessel, now very much narrowed, but with a patent lumen, runs down into the ventricular muscle anterior to and to the left of the aorta. In the muscle it ends as a blind smooth endothelial-lined pocket to the right of the anterior descending coronary artery. Neither valves nor any continuation as a fibrous cord into the ventricular cavity can be made out.

The left atrium measures 15 mm in transverse and 20 mm in vertical diameter. The openings of the pulmonary veins are normally situated. The patent foramen ovale is evident. The left atrioventricular orifice with the bicuspid valve and its papillary muscles is not remarkable. It measures 20 mm in circumference. The anterior leaflet bounds the left base of the aorta and is in the usual intimate relation to the left aortic cusp. The left ventricle is much less heavily trabeculated than the right and measures 30 by 10 mm, in contrast to 30 by 20 mm for the internal measurements of the right ventricle. The left coronary artery arises from the left posterior aortic sinus, while the right coronary artery arises from the anterior aortic sinus. The left coronary artery passes behind the atretic pulmonary artery and emerges on its left side. The thickness of the right and of the left ventricular myocardium is 5 mm.

These 5 cases present a series of type 1 transpositions in which the aorta moves from a position over the left ventricle to one over the right. Hypertrophy of the right ventricle, subaortic interventricular defects and a rotation of the entire heart (so that the usual anterior surface is turned to the left side) are also present. All the examples save that in case 3 show either a rudimentary or a bicuspid condition of the pulmonary valve while all except that in case 5 clearly show hypertrophy and counterclockwise rotation of the crista supraventricularis. In case 2 the crista is sufficiently well developed to form an accessory septum, which separates a pulmonic ventricle from the remainder of the right chamber. The course of the coronary arteries is normal except for the fact that the coronary-bearing aortic sinuses are rotated in a counterclockwise direction. The condition of the ductus arteriosus and interatrial septum varies considerably. A persistent foramen ovale occurs once, while patency of the ductus arteriosus is present in 4 cases. In case 4 there is a persistent ostium primum accompanied by deformities of the atrioventricular valves described by Abbott and Kaufmann and by Gunn and Dieckmann. A mild degree of aortic coarctation is seen in case 3. Case 1 shows a complete vascular circle about the trachea and esophagus because of the abnormal persistence of the fourth right aortic arch and the ductus arteriosus.



The relatively slight degree of detorsion postulated by Spitzer as the etiologic factor of this type of transposition is verified by the diminished amount of spiraling of the great vessels and by the counterclockwise rotation of the entire heart, coronary orifices and crista supraventricularis. Theoretically, the proximal portion of the septum aorticum has disappeared, so that the right ventricular aorta has opened and fused with the usual left ventricular aorta. The result is an overriding aorta and septal defect between the anterior bicuspid ledge and the crista supraventricularis in that portion of the septum which is derived from the septum aorticum (the posterior portion of the anterior septum of Rokitsansky). The pulmonary artery is stenotic and bicuspid for the reasons already discussed on page 453. The limit of this process is reached in the complete obliteration of the pulmonary artery in case 5, probably because of the fusion of the crista supraventricularis with the anterior portion of the real septum ventriculorum. These two, therefore, form a composite anterior interventricular septum in this case.

#### TRANSITIONAL FORM BETWEEN TYPE 1 AND TYPE 2

*CASE 6—Torsion of the entire heart, right ventricular hypertrophy, intact interatrial septum, interventricular septal defect, patent ductus arteriosus, overriding aorta, stenotic pulmonary artery, single coronary artery orifice*

A 7 month old girl had shown labored respirations, slight cyanosis and poor weight gain since birth. Development was slightly retarded. There were occasional attacks of edema of the extremities lasting several days each. Markedly increased cyanosis and dyspnea occurred during the three days prior to death. Physical examination showed malnutrition, poor development, cyanosis, dyspnea, cardiac enlargement to the right and to the left, a rough systolic murmur over the precordium heard also at the aortic area and transmitted to the back, clubbing of nails and cold extremities. The red blood cell count was 6,880,000. At autopsy the following additional observations were made: interstitial bronchopneumonia, generalized visceral congestion, cerebral edema. Microscopic examination of the heart showed moderate congestion and edema. Occasionally muscle fibers stained irregularly.

The heart is moderately enlarged, weighing 50 Gm (normal weight, 34 Gm). It measures 5 cm in the vertical diameter, 7.5 cm in the transverse diameter and 7.5 cm in the oblique diameter. The internal diameter of the thorax is 11 cm. The shape of the heart is not abnormal, and the apex points downward and slightly to the left. The anterior interventricular groove begins close to the left border and runs downward and to the left to reach the obtuse margin 1.5 cm above the apex. The posterior groove also courses down to the left after beginning at the middle of the ventricular base. Thus the anterior surface of the heart is made up almost entirely of the right ventricle, and the interventricular septum assumes a more coronal position than usual. The right atrium is brought forward by the twisting of the whole heart and projects to the right of the large aorta, which arises midway between the middle of the base and the left border. The very small pulmonary artery is not seen anteriorly. It lies beneath the aortic arch, reaching the ventricles at the anterior interventricular groove, where it lies behind and to the left of the aorta.

The right atrium is large, and the trabeculation, in front of the crista terminalis, is coarse and hypertrophied. The orifices of the veins and the venous valves are normally situated. The fossa ovalis measures 3 by 6 mm and is functionally closed. The right atrioventricular orifice has a circumference of 55 mm and bears anterior, posterior and medial leaflets. The papillary muscles are large and normally situated. The aorta arises from the extreme anterior and medial portion of the right ventricle. Immediately behind and medial to the aortic base is a moderate-sized ledge which arches over the ventricular base from the posterior border of an interventricular septal defect to the anterolateral right ventricular wall. This ledge separates the aorta from the tricuspid valve, to which it is closely applied. The septum ventriculorum presents an oval defect, measuring 7 by 4 mm in diameter, at its anterior upper portion, which is directly beneath the posterior half of the left side of the aorta. From the anteroinferior margin of the defect a mass of trabeculations, 18 cm wide and 25 cm long, runs down the right side of the septum and then turns anterolaterally to reach the anterolateral ventricular wall, where it gives rise to the anterior papillary group of muscles. The medial papillary muscle arises from the medial portion of the posterior margin of this ledge.

The aortic valve measures 35 mm in circumference and has right lateral, left anterior and left posterior cusps. From the sinus of the latter opens the single coronary artery, which immediately branches. The left branch winds behind and around the left side of the pulmonary artery to reach the anterior longitudinal groove, where it forms the anterior descending branch. The right branch winds about the aorta to reach its right side and then courses in the posterior coronary sulcus, where it ends as the posterior descending artery.

The left atrium, the orifices of the pulmonary vein and the left atrioventricular valve are not remarkable. The valve is bicuspid, measures 30 mm in circumference and is connected to markedly hypertrophied papillary muscles in their usual position. The base of the medial leaflet runs up through the defect in the interventricular septum to the left posterior cusp of the aorta. The opening of the pulmonary artery into the ventricles cannot be seen. On opening the pulmonary artery toward the ventricle there is disclosed a valve measuring 15 mm in circumference and bearing three small delicate cusps situated posteriorly and anteriorly on the right and on the left. For 3 mm below the valve the pulmonary artery courses through muscle toward the left, to end in a pocket which communicates through a pin-sized opening with the extreme anterior portion of the left ventricle. Thus the pulmonary artery, which at first appears to be directed to the right ventricle (to the right of the anterior descending coronary artery), courses through the musculature to end in the left ventricle. The branches of the great vessels are not remarkable. The ductus arteriosus admits a 1 mm probe.

This case demonstrates the manner in which the next two types may be formed. The aorta now arises almost entirely from the right ventricle, and the septal defect is unusually small. In accordance with the variations shown thus far by the series of cases, one would next expect the total emancipation of the aorta from the left ventricle. This implies obliteration of the left ventricular aorta and the complete assumption of its functions by the reopened right ventricular aorta. The resulting simple transposition of the aorta from the left to the right ventricle is characteristic of type 2 transposition.

In the first series of cases the pulmonary artery has become progressively narrower because of the more sagittal direction and hypertrophy of the crista supraventricularis. The limit of this process was seen in case 5, where the crista and the true anterior septum had fused to a composite septum. If, now, the true anterior septum should regress, the crista supraventricularis might take over its functions, since it is placed in the usual position of the anterior septum and since it has the necessary septum-building potentialities, as demonstrated in case 2.

In case 6 a heavy ledge courses down the right side of the interventricular septum between the pulmonary artery and the aorta. Other theories cannot account for the presence of this band of trabeculations in the conus arteriosus of the right ventricle. According to the theory of Spitzer, the crista supraventricularis should occupy this position between the great vessels. The anterior interventricular septum in this case must therefore be a composite one, made up of the crista to the right of, and the true anterior septum to the left of, the markedly stenotic pulmonary artery. The partial regression of the left portion is shown by the termination of the artery in the left ventricle. Complete regression would place the artery entirely in a left ventricle, giving rise to a type 3 transposition. An indication of the probability of these deductions is present in the position of the anterior descending coronary artery, which marks the left border of the composite septum and is situated to the left of the pulmonary artery. Moreover, on the right, the inferior portion of the septal ledge courses, as the trabecula septomarginalis, to the anterior papillary muscle of the tricuspid valve.

The muscle band which is situated between the aorta and the anterior tricuspid leaflet resembles the normal crista supraventricularis and has frequently been assumed to be this. However, it is not homologous to the crista. The reasons for this and Spitzer's explanation of its origin from the right portion of the bulboatrial ledge have already been given.

#### TYPE 2 SIMPLE TRANSPOSITION OF THE AORTA

*CASE 7 (fig 8) —Marked hypertrophy of right atrium and ventricle, persistent ostium primum, atresia of mitral valve, aplasia of left ventricle, normal pulmonary artery, atresia of proximal portion of aorta, formation of the coronary arteries and aortic arch from ductus arteriosus, marked hypertrophy of crista supraventricularis to form accessory pulmonary chamber, abnormal coronary sinus, fetal endocarditis of the mitral valve*

An 8 day old boy appeared normal until twelve hours before death, when he began to suffer from labored respirations and severe cyanotic attacks. Physical examination showed cyanosis, cold extremities, signs of pneumonia at the bases of the lungs and a systolic murmur, best heard at the base of the heart. At autopsy the following additional observations were made: terminal bronchopneumonia, acute splenitis and visceral congestion.

Sections through the atretic mitral valve show marked thickening of the endocardium. The normal course of the fibrous bundles is distorted, and the endocardium

is unusually cellular. Numerous collections of lymphocytes, interspersed with neutrophils and occasional eosinophils, are scattered in the neighborhood of the valve and the adjacent coronary sulcus. Many of these collections are in close relation to the blood vessels, which are moderately congested. The tissue of the coronary sulcus and the base of the valve are unusually well vascularized. The left ventricular endocardium is not remarkable save for thickening. Sections through the right and the left ventricular myocardium show only moderate congestion and slight edema.

The heart weighs 32 Gm (normal weight, 18 Gm). It is roughly semicircular in shape and lies with its long axis to the left. The acute margin is long and curved, forming the right lateral, inferior and 5 mm of the lower left border. The short obtuse margin runs down and to the left. The anterior and posterior coronary vessels course down very close to the left border, cutting off a small segment of the heart, this measures approximately 5 mm in diameter. Thus, externally, the heart seems to consist principally of right ventricle. The upper

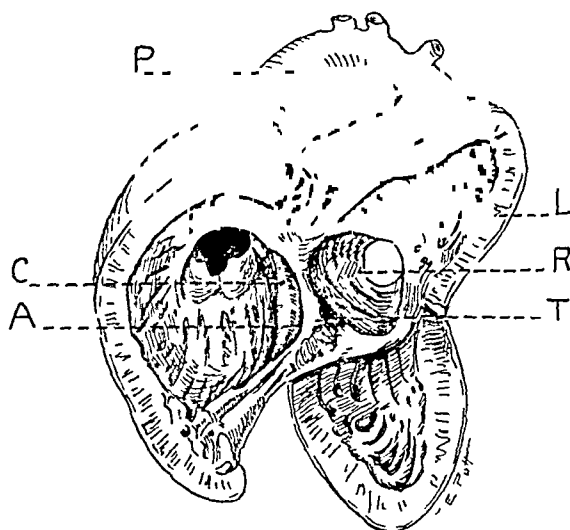


Fig 8 (case 7) —The ventricular cavity is viewed from below and anteriorly. *P* indicates the pulmonary artery, *A*, the point of origin of the aortic valve, *C*, the crista supraventricularis, *T*, the tricuspid valve, *R*, the right atrium, and *L*, the site of the slitlike left ventricle in the cardiac wall.

anterior portion of the heart is bulged forward to form a prominence which gives rise to a large pulmonary artery situated in the center of the cardiac base. The aorta cannot be seen. The atria are entirely on the right side of the heart save for a small appendage which projects forward to the left of the pulmonary artery.

The right atrium is a very large chamber, measuring 35 by 25 mm, in contrast to 15 by 10 mm for the left atrium. Its walls are 2 mm thick and are heavily trabeculated. The superior and inferior venae cavae open into the extreme posterior and left portion of the right atrium, opposite one another. The eustachian valve divides into a very small semilunar valve guarding the orifice of the coronary sinus and a portion is inserted into the inferior part of the interatrial septum. The orifice of the coronary sinus is of pinpoint size. The coronary sinus divides immediately into a small coronary sinus proper, a small posterior interventricular vein and a much larger right cardiac vein. The last enters the atrium through an unguarded 3 mm orifice at the anterolateral border just

beneath the auricular appendage. Apparently the right cardiac vein has taken over the function of the coronary sinus and opens independently into the atrium. The interatrial septum is present as a crescentic structure on the posterior and inferior walls of the atria. It runs obliquely from a left posterior to a right anterior position and leaves a large defect anteriorly, measuring 33 mm in circumference. The atria are functionally a single cavity. In the posterior remnant of the septum, above the insertion of the eustachian valve, is a small oval depressed area, measuring 3 mm in diameter, which is guarded by a slightly raised edge anteriorly and inferiorly. In the base of this depression is a pinhead-sized opening which leads past a crescentic flap on the left side of the septum to open into the left atrium. This area corresponds in position and form to the true fossa ovalis. The large interatrial defect is an accessory foramen, probably a persistent ostium primum. The right atrioventricular orifice measures 17 mm in diameter. The cusps, of approximately equal size, are situated in anterior, lateral and medial position. The anterior and posterior papillary muscles are normal. The medial cusp is bound directly by chordae to the left wall of the ventricle. The large anterior portion of the right ventricle gives rise to the pulmonary artery at its summit. A very heavy ledge, 1 cm deep, runs from the left anterior wall of the ventricle backward, upward and laterally behind the pulmonary orifice and in front of the anterior tricuspid leaflet to reach the lateral wall of the ventricle, where it is lost in the region of the anterior papillary muscle. From the anterior papillary muscle another moderately large ledge runs along the inferior portion of the ventricle, opposite the first ledge, to reach the left wall of the ventricle close to the origin of the first ledge. The former corresponds in position to the crista supraventricularis and the latter to the trabecula septomarginalis. Together they form an abortive septum which separates the outflow from the inflow portions of the right ventricle. The pulmonary artery measures 10 mm in diameter at the valve, which contains posterior, left and right cusps. The pulmonary artery courses straight upward, giving off the right and the left branch, and is then continued into a wide ductus arteriosus to form the descending porta. Proximal to this junction there is a small vessel which courses down toward the ventricle to form the arch of the aorta, which gives off the usual vessels. It is intimately connected to the pulmonary artery by fibrous tissue and at the ventricular base is located right and posterior to that vessel. Near the heart the small vessel bifurcates into two coronary arteries. From the bifurcation a small fibrous cord runs into the right ventricular muscle to become lost in the region between the crista supraventricularis and the anterior tricuspid leaflet.

The left atrium is small, measuring 15 by 10 mm. It receives the four pulmonary veins at its medioposterior portion. The only outflow from this atrium is through the interatrial defect into the right atrium. The left atrioventricular orifice is represented by a small dimple in the floor of the left atrium. Beneath this dimple, in the left wall of the single ventricle, is a blind endothelium-lined space, which measures 1 by 2 mm in diameter and 5 mm in length. The portion of the left wall of the heart (and right ventricle) which contains this space is demarcated from the rest of the ventricular walls by the anterior and posterior descending coronary arteries. This small space, therefore, represents a left ventricle which has been almost completely obliterated. The only ventricle which is functioning has the configuration of the normal right ventricle. The niche between the crista supraventricularis and the anterior tricuspid leaflets, however, leads not to a blind pocket but to a fibrous cord marking the beginning of the atretic aorta. The coronary arteries have essentially the normal course and distribution.

The heart in case 7 is an example of a *cor biatiatum* pseudo-triloculare resulting from atresia of the mitral valve and almost complete disappearance of the left ventricle. The remaining chamber should show the various structures of the right ventricle with unusual clarity, and the configuration of that chamber should be theoretically explicable as the result of the disappearance of *all* left ventricular formations, including the normal aorta. Actually the heart shows marked hypertrophy of the crista supraventricularis and trabecula septomarginalis. These form a partial septum behind the pulmonary artery. The conus and pulmonary valve are apparently normal. However, the niche between the crista supraventricularis and the anterior tricuspid valve leaflet leads not to an empty pocket as in the normal heart but to a fibrous cord which marks the origin of the atretic aorta.

It has formerly been assumed that an early closure of the mitral valve would cause the aorta to seek an entrance into the right ventricle because of the preaortic deviation of blood. The sequence of events can be partly explained by the theory of Rokitsky. A displacement of the ventricular septum to the left end of the fused atrioventricular cushions because of the left ventricular atrophy incident to the mitral atresia might prevent the final transfer of the aorta from the right to the left ventricle. However, since the theory implies an independence of the truncus and ventricular septums, a deviation of the ventricular septum alone should not change the position of the truncus septum and cause the aorta to assume a position posterior to, and to the right of, the pulmonary artery. One would expect to find both trunks arising from the right ventricle in *normal* relation to each other. A similar argument is valid even though one postulates an eccentric position of the truncus septum as a result of the diminution of blood flow through the aorta.

The explanation, according to the theory of Spitzer, is so obvious as to make the condition appear to be one of nature's excellent experiments. The defect in the interatrial septum and the disappearance of the left ventricular aorta follow the mitral atresia because of the mechanical changes in blood flow. The obliterative process in the right ventricular aorta should cease, since that vessel must remain open to take care of the markedly increased blood flow through the right ventricle. For the same reason, the pulmonary artery should be wide and the valve tricuspid, in contrast to the usual stenotic conditions in type 1 and type 2 transposition.

The details of the final structure of the heart would depend on the time in development at which the mitral atresia or stenosis occurred. If this took place before the obliteration of the right ventricular aorta, the result should be a simple transposition of a patent aorta. Atresia occurring when the right aorta was almost entirely closed should cause

simple transposition of an atretic aorta. This is shown by case 7. Mitral atresia arising after this time should result in an atretic or patent aorta in normal position<sup>14</sup>

Two examples of mitral atresia combined with simple transposition of a patent aorta were found in the collection at the Children's Hospital. These illustrate the first of the four possibilities mentioned.

CASE 7a—A 9 day old boy showed micrognathia, cleft palate, bilateral megalo-ureters and numerous other congenital anomalies.

The aorta, measuring 22 mm in circumference at the valve, arises to the right of the somewhat larger pulmonary artery. Both great vessels issue from a markedly enlarged right ventricle. The interior of the right ventricle shows a crista supraventricularis in front of the aorta, separating the latter from the pulmonary artery. Behind the aorta is the anterior leaf of the tricuspid valve. The right atrium is three times as large as the left and communicates with it through a foramen ovale, measuring 3 by 7 mm. The left ventricle is a small slitlike space in the wall of the heart. It lies directly beneath a small dimple in the floor of the left atrium, which represents the atretic mitral valve. The two ventricles communicate through a 1 mm defect opening into the most posterior portion of the right ventricle. Proximal to the insertion of the ductus arteriosus there is a marked degree of aortic coarctation.

CASE 7b—The patient was a 4 day old girl with bilateral choanal atresia.

The heart is almost an exact duplicate of the preceding example. The positions of the vessels and the relative dimensions of the atria and ventricles are similar. The foramen ovale measures 1.5 cm in diameter. Each of the great vessels has a circumference of 20 mm. The aorta has the same position between the crista supraventricularis and the anterior leaflet of the tricuspid valve. In addition to a defect leading from the slitlike left ventricle into the posterior portion of the right ventricle, there is another 1 mm defect which leads into the subaortic region on the right.

In the foregoing discussion it has been assumed that the appearance of the mitral anomaly preceded the changes in the aorta. This assumption is strengthened by an examination of the incidence of the two anomalies. It is well known that mitral atresia is very uncommon as compared with transposition of the great vessels. If the two anomalies were totally unrelated, one would expect the combination to be extremely

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<sup>14</sup> Kung reported a case of mitral atresia with an atretic left-chambered aorta and criticized Spitzer's theory on the ground that the right ventricular aorta should have reopened. It is interesting to note that Spitzer answered the objection by maintaining that only through detorsion, which places the heart in an earlier phylogenetic position, can the atavistic right ventricular aorta be reopened. Mitral atresia is not necessarily associated with such detorsion. We prefer the explanation given in the foregoing text, however, since all the anticipated variations in the position of the aorta have been reported. (The cases of Jost, Frankel and Monckeberg, and Donnally show the aorta in normal position.) It must be noted that the time of obliteration of the right ventricular aorta refers not to actual closure of the vessel but to the stage in ontogeny after which detorsion can no longer lead to the development of that aorta.

rare indeed. Furthermore, if transposition preceded and caused the mitral anomaly, a large proportion of cases of transposition should show mitral atresia. Neither conclusion is true. On the contrary, it is found that in an unusually large proportion of cases mitral atresia is associated with some form of transposition, 8 of 12 cases reviewed by Dudzus and 6 of an additional 13 cases recorded in the literature definitely showed transposition of the aorta in association with mitral atresia.<sup>15</sup> The logical assumption is, therefore, that the marked frequency of this association is due to a causative role of the mitral atresia.

In addition, case 7 shows definite fetal endocarditis in the neighborhood of the mitral valve. Unfortunately, it was impossible, either from the microscopic examination or from the history of the pregnancy, to determine the age of the inflammatory process. For this reason, no statement concerning the relation of the endocarditis to the transposition or to the mitral atresia can be made, although speculation naturally turns to the possibility of mitral endocarditis acting as the first in the chain of events leading up to the developmental anomaly of transposition. No report of a similar combination of definite nonsyphilitic fetal endocarditis and transposition could be found.

#### TYPE 3 COMPLETE TRANSPOSITION OF THE GREAT VESSELS

*CASE 8—Hypertrophy of heart, intact interatrial septum, small interventricular septal defect, patent ductus arteriosus, complete transposition of great vessels, normal pulmonary valve, rotation of coronary-bearing aortic sinuses*

A 4½ month old boy was well until the age of 2 months. He then began to have feeding difficulties and failed to gain weight. After that he showed dyspnea, cyanosis, cardiac hypertrophy, precordial systolic murmur and mild hepatomegaly. At autopsy the following additional observations were made: acute interstitial bronchopneumonia, multiple hemorrhages in the right parietal cerebral cortex and chronic passive congestion of the viscera. Microscopic examination of the heart showed moderate interstitial edema and slight degenerative changes in the subendocardial myocardium.

The heart is rounded and is greatly enlarged in all diameters. The apex is blunt and points downward and slightly to the left. The posterior interventricular groove is well marked, running down and to the left. Three coronary arteries descend on the anterior surface of the heart. The main coronary artery passes downward and to the left, reaching a point just to the left of the apex, 8 mm. to

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<sup>15</sup> In the analysis of cases of mitral atresia it is necessary to include those hearts showing bulboventricular inversion and atresia of the right (mitral) atrioventricular orifice. The cases reported by Hedinger, Gaspar, and Abbott and Moffatt fall into this category. Conversely, examples of bulboventricular inversion with atresia of the left (tricuspid) atrioventricular valve must be excluded. The case of Hu and possibly that of Dudzus show this condition (see also case 17 of this series). As pointed out by Monckeberg, the final determination of the inversion in doubtful cases must rest on the character of the atrioventricular bundles, since the anterior leaflet of a mitral valve may be split and thus make the valve appear tricuspid.



the right of this is the middle, almost equally large artery, which runs down parallel to the first. The right artery is the smallest. It is 6 mm to the right of the second but extends only three quarters of the way down the surface of the ventricles. On opening the heart it is found that the first artery is situated to the left of the interventricular septum, that the second is directly over it and that the third is slightly to the right of the septum. The aorta arises to the right of all three coronary arteries, while the equally large pulmonary artery arises from the space between the middle and the left coronary artery, posterior and to the left of the aorta. The aorta and the pulmonary artery pursue parallel courses directly upward.

The right atrium and right ventricle are more prominent than usual. The foramen ovale is functionally closed but shows a small slitlike opening anteriorly. The venous valves are well marked, and the veins enter in their normal positions. The right atrioventricular valve is tricuspid. The medial cusp is larger than usual, but no medial papillary muscle can be seen. The attachment of the medial cusp is directly to the interventricular septum. The other papillary muscles are not remarkable.

The tricuspid valve measures 55 mm in circumference, and the right ventricular wall measures 6 to 7 mm in thickness. The aorta arises from the extreme left anterior and superior portion of the right ventricle. Beginning on the interventricular septum is a ledge (*A*)<sup>16</sup> which passes posterior to the aorta and anterior to the tricuspid valve to run laterally across the ventricular wall and be inserted at the origin of the anterior papillary muscle. Between this and the anterior tricuspid leaflet is a niche which leads up to a 3 by 4 mm defect in the upper midportion of the interventricular septum, corresponding in position to the usual location of the membranous septum. The aortic valve has three normal cusps—right anterior, posterior and left anterior. The sinuses of the latter two bear the coronary orifices. From the posterior cusp a poorly defined band of trabeculations (*T*)<sup>16</sup> courses vertically down the septum to branch and becomes lost in the midportion of the septum. This crosses anterior to the ledge previously described.

The left atrium, pulmonary veins, bicuspid valve and papillary muscles are not remarkable. The pulmonary artery arises from the extreme anterior and right portion of the left ventricle. It contains normal right posterior, left posterior and anterior cusps. The pulmonary valve measures 30 mm in circumference, in contrast to the aortic measurement of 25 mm. Five millimeters below the right posterior cusp of the pulmonary valve is the interventricular defect. The medial cusp of the mitral valve has the intimate relation to the posterior cusp of the pulmonary artery as it normally has with the corresponding cusp of the aorta. A band, measuring 2 mm in width, courses on the left side of the septum from just below the interventricular defect, horizontally and slightly downward, to the anterior ventricular wall. This demarcates a smooth superior portion of the septum from a rougher inferior area. Above the left anterior cusp of the aorta are two coronary openings. The one to the right gives rise to the extreme right, or third, anterior descending coronary artery, previously described. The latter gives off a right circumflex branch which passes in front of the aorta and ends in the posterior descending artery. The left orifice in the left anterior aortic sinus leads only to the middle of the three anterior descending arteries. The vessel from the posterior aortic sinus winds around behind the pulmonary artery to reach the coronary sulcus between the pulmonary artery and the left atrium. Here

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16 See discussion, pages 446 and 447

it branches into a left circumflex artery, which passes back to the right in the posterior coronary sulcus, and to the largest, left anterior descending artery. The branches of the great vessels are not remarkable. The right pulmonary artery passes beneath the aorta. It gives off a ductus arteriosus which admits a 1 mm probe.

*CASE 9—Cardiac hypertrophy, open foramen ovale, small interventricular septal defect, closed ductus arteriosus, complete transposition of great vessels, rotation of coronary orifices, partial transposition of coronary arteries*

An 8 month old boy had cyanotic attacks and failed to gain satisfactorily from the time of birth. Physical examination showed dilated veins in the scalp, deep ulcers in the buttocks, slight cardiac enlargement, precordial systolic murmur, undescended testicles and spastic diplegia. At autopsy the following additional conditions were observed: atelectasis and fibrosis of the right lung, chronic bronchopneumonia, multiple pulmonary abscesses, visceral congestion, thrombophlebitis of the longitudinal and left sigmoid sinuses and infarction of the right cerebral hemisphere. Histologically the heart was found not remarkable.

The heart is enlarged, measuring 8 cm in its greatest transverse diameter, in contrast to the transthoracic diameter of 12 cm. The anterior interventricular groove is closer to the left border of the heart than usual. The posterior groove is also nearer the left border, so that the right ventricle appears to be much larger than the left. The upper anterior portion of the right ventricle shows a rounded prominence from which the aorta arises. It is approximately in the center of the cardiac base. The pulmonary artery is posterior to and a little to the left of the aorta. Both vessels are of approximately equal size and course straight upward.

The right atrium is greatly dilated. The veins enter in their usual position, but the venous valves are rudimentary. The fossa ovalis is large, measuring 17 by 20 mm. The anterior wall of the limbus is well marked, but the posterior is barely discernible. The fossa is closed posteriorly by a septum which has a free crescentic anterior border, leaving a defect in the interatrial septum, measuring 2 by 14 mm. This space is divided by a small band. The right atrioventricular opening is large, measuring 50 mm in circumference. It bears three cusps of almost equal size. The papillary muscles are not remarkable. In the postero-superior portion of the muscular interventricular septum is an oval defect, measuring 6 by 4 mm. This is situated below the medial tricuspid leaflet in the posterior, or inflow, portion of the right ventricle. The defect leads into the posterior part of the left ventricle at a point two thirds of the distance from the apex to the base. One centimeter above and slightly anterior to the defect is an intact membranous septum, which is in the usual position. The defect is therefore in the posterior septum of Rokitansky. The aorta enters the anterior portion of the right ventricle in the region of the prominence noted on the surface. The circumference of the valve is 30 mm, and the cusps are situated in posterior, left anterior and right anterior positions. The posterior base of the aorta is defined by a moderate-sized ledge (*A*)<sup>16</sup> which begins at the anterior border of the interventricular septal defect, arches across the right ventricular base just behind the aorta and then runs laterally to reach the anterior papillary muscle. It courses in front of the anterior tricuspid leaflet, leaving a shallow niche which leads up to the undefended space of the septum. Beginning at the medial side of the base of the aorta, a heavy band (*T*)<sup>16</sup> courses down the septum before turning laterally to reach the anterior papillary muscle of the tricuspid valve. The band is 10 mm wide, 6 mm deep and 24 mm long. The posterior portion of this wide ledge gives rise to a large medial papillary muscle and to numerous chordae to the anterior and

medial tricuspid leaflets The ledge is massive, and only a small space remains between it and the group of papillary muscles through which the blood must pass to reach the aorta

The left atrium, pulmonary veins, mitral orifice and bicuspid valve are not remarkable The anterior papillary muscle is inserted toward the left lateral wall, so that the valve runs more diagonally left and forward than usual The interventricular septal defect may be seen in front of the posterior papillary muscle which arises at the angle between the septum and the posterior wall of the ventricle The pulmonary artery takes origin from the anterosuperior portion of the left ventricle and is guarded by three valve cusps situated in anterior, right posterior and left posterior positions Below the right posterior cusp is the membranous septum The anterior and left posterior cusps are in intimate relation to the medial cusp of the mitral valve The branches of the aorta and pulmonary artery are not remarkable The ductus arises from the left pulmonary artery but is not patent One coronary artery arises from the space above the junction of the posterior and left anterior aortic cusps It runs forward and to the left between the aorta and pulmonary artery It then turns to the right in the anterior coronary sulcus and branches to give small vessels which descend over the anterior right ventricular wall and a small right circumflex artery which gradually disappears in the coronary sulcus In the posterior aortic sinus is a very large coronary orifice This coronary artery divides almost immediately into a left superior and a right inferior branch The former winds around behind the pulmonary artery and then passes in front of it to form the anterior descending coronary artery It also gives off the left circumflex artery which supplies the posterior portion of the left ventricle The right inferior branch forms a large right circumflex artery which appears in the coronary sulcus between the aorta and right atrium It supplies the posterior surface of the right ventricle and ends in the posterior descending artery The anterior descending coronary artery is situated just to the left of the massive interventricular septum

*CASE 10—Cardiac enlargement, open foramen ovale, minute interventricular septal defect, closed ductus arteriosus, complete transposition of great vessels, rotation of coronary artery orifices, partial transposition of coronary arteries and mild degree of aortic coarctation*

A 35 day old boy showed extreme cyanosis at birth and severe attacks of cyanosis till death A faint systolic murmur was first heard at the age of 3 weeks The murmur increased in intensity until death For two weeks prior to death the patient showed peripheral edema, pulmonary congestion and severe cyanosis The autopsy was limited to a gross examination of the heart

This heart is very similar to that described in case 8 It is large and rounded, with a blunt apex Two coronary arteries, of moderate size, run down the anterior surface of the ventricles close to the left border The left and larger of these two arteries bounds the left side of the interventricular septum, while the smaller, right vessel is situated over the right side of the septum The posterior interventricular sulcus and the descending branch of the coronary artery run down the middle of the posterior aspect of the heart The aorta emerges from a prominence at the medial superior portion of the anterior aspect of the right ventricle To the left of the aorta and posterior to it is the pulmonary artery Both arteries are small and course directly upward

The right atrium is large, measuring 15 by 15 mm The venae cavae, coronary sinus, eustachian valves and thebesian valves are normal The foramen ovale is closed by a membrane, which is latticed in its anterior portion and which affords

a small entrance into the left atrium, 8 mm in diameter. The tricuspid valve measures 35 mm in circumference and bears three cusps in normal positions. The aorta arises from the outpocketing of the right ventricle previously noted. This pouch and the posterior base of the aorta are demarcated by a ledge (*A*)<sup>16</sup> which arises high up on the septum medially and arches across the base of the ventricle posterior to the aorta. It then turns laterally to reach the anterior papillary muscle of the tricuspid valve. Between this and the anterior tricuspid leaflet, which lies directly behind it, is a niche which leads up to a pinpoint opening in the ventricular septum. The aortic valve measures 18 mm in circumference and contains an anterior, right posterior and left posterior cusp. Above the latter two cusps are the coronary orifices. Extending down from the left posterior cusp is a trabecular band (*T*)<sup>16</sup> which courses down the interventricular septum and crosses the origin of the ledge previously described. It disappears approximately two thirds of the way down the septum.

The left atrium receives the four pulmonary veins and leads into the ventricle through a normal opening guarded by a bicuspid atrioventricular valve, 35 mm in circumference. The pulmonary artery arises from the anterior right portion of the left ventricle and is guarded by a tricuspid valve. The cusps are situated in posterior, left anterior and right anterior positions. The anterior cusp of the mitral valve is separated from the pulmonary artery by a small area of muscle on the ventricular base. Below the right side of the pulmonary artery is the pinpoint-sized interventricular septal defect. The remainder of the septum is smooth and intact. The coronary artery arising from the left posterior aortic sinus gives rise to the two anterior descending coronary arteries and to a left circumflex branch, all of which course anterior to the pulmonary artery. The other main coronary artery arises in the right posterior aortic sinus. It forms the right circumflex branch and ends as the posterior descending artery. The aorta narrows to a circumference of 14 mm after it gives off the three main branches from the arch but widens to a diameter of 16 mm distal to the ductus. The ductus arteriosus is not patent.

*CASE 11—Marked cardiac hypertrophy, functionally closed foramen ovale, intact interventricular septum, patent ductus arteriosus, complete transposition of great vessels, rotation of coronary artery orifices and complete transposition of the coronary arteries*

A 4 week old girl had difficulty in feeding, a feeble cry and cyanotic attacks from the time of birth. Several hours before death, marked cyanosis, orthopnea and dyspnea appeared. Physical examination revealed cyanosis, tachycardia, cardiac enlargement to the left, gallop rhythm, mild hepatomegaly and cold extremities. No murmurs were heard.

At autopsy the following additional observations were made: acute interstitial bronchopneumonia with areas of atelectasis and emphysema, fatty infiltration of the liver and general visceral congestion. Microscopic examination of the heart showed marked congestion, mild scattered vacuolation and a coarse granular appearance of muscle fibers. The endocardium was found normal.

The shape of the heart is approximately normal. The measurements are 6.5 cm in vertical diameter, 5 cm in horizontal diameter and 9 cm from base to apex. It lies almost transversely in the thoracic cavity, and the anterior interventricular groove runs downward and to the left. The right auricular appendage is very prominent at the right superior portion of the heart.

The right atrium is not remarkable except for hypertrophy. It receives the venae cavae and the coronary sinus in normal positions. The interatrial septum

is intact The right atrioventricular orifice measures 42 cm in circumference and bears normal anterior, medial and posterior cusps The anterior papillary muscle is large and bifid The aorta arises as a large vessel from the left anterior portion of the right ventricle Behind and to the left of this is the equally large pulmonary artery, which leaves the left ventricle Both arteries course directly upward The aortic valve measures 28 mm in circumference and bears right posterior, left posterior and anterior cusps A moderately heavy muscle ledge takes origin from the right side of the interventricular septum and passes upward and laterally behind the aorta to reach the right anterior portion of the ventricular wall near the insertion of the anterior papillary muscle (*A*)<sup>10</sup> From the anterior papillary muscle of the tricuspid valve a muscle band runs down toward the apex and then over to the septum to join it two thirds of the way from the apex to the base (*T*)<sup>10</sup> It then proceeds upward on the septum to reach the left posterior cusp of the aorta These two ledges form the boundary between the anterior or aortic portion of the right ventricle and the posterior or tricuspid portion The interventricular septum is intact and contains a completely closed membranous portion in the same position in relation to the pulmonary artery and tricuspid valves as it normally has to the latter and the aorta

The left atrium is smaller than the right It receives the four pulmonary veins in normal positions and leads into the ventricle past a bicuspid valve, measuring 35 mm in circumference The cusps are normally situated The pulmonary artery arises from the anterior right portion of the ventricle The valve measures 30 mm in circumference and has right, left anterior and left posterior cusps One coronary artery arises in the right posterior aortic sinus and emerges between the aorta and the right atrium to form the right circumflex and posterior descending arteries The second coronary artery arises above the left posterior aortic cusp and branches in front of the pulmonary artery to form the anterior descending artery, the left circumflex and several small branches which course down the surface of the left ventricle The anterior descending artery is situated directly over the interventricular septum The pulmonary artery and the aorta give off the usual branches They are joined by a short patent ductus arteriosus, which has a diameter of 4 mm

Cases 8, 9, 10 and 11 are examples of type 3 transposition, in which the aorta emerges from the right ventricle and the pulmonary artery from the left The greater degree of detorsion postulated for this type is evident in the decreased spiraling of the great vessels and the greater counterclockwise rotation of the orifices of the coronary arteries The left ventricular aorta has been obliterated, and the right aorta has reopened As a result of the counterclockwise detorsion the crista supraventricularis has been placed in the usual position of the true anterior interventricular septum The crista thus lies in the same plane as the posterior portion of the true anterior interventricular septum The crista has, therefore, hypertrophied to form a false anterior septum The anterior portion of the true septum has disappeared, and the pulmonary valve has regained the tricuspid condition

These cases show a peculiar ledge which courses down the right side of the septum from the base of the aorta In the description, this ledge has been designated by the letter *T* It appears that a clue to its nature

is given by case 11. Here the ledge is more clearly defined and is seen to run from the anterior papillary muscle of the tricuspid valve to the posterior superior portion of the false anterior septum. The latter position theoretically corresponds to the original medial or septal margin of the crista supraventricularis in the normal heart. Thus, the ledge actually follows the course of the trabecula septomarginalis proper from the anterior papillary muscle of the tricuspid valve to the former septal origin of the crista supraventricularis. It courses down and completes the false septum. The shift to a more sagittal and vertical direction of the trabecula would result from the counterclockwise detorsion of the heart.

The ledge marked by the letter *A* in the text separates the right ventricular aorta from the anterior tricuspid leaflet. It is thus in the position between the portions of the ventricles derived from the ascending and descending primitive loops. According to Spitzer, it is the hypertrophied remnant of the bulboatrial ledge. The varying degrees of transposition of the coronary arteries will be discussed later.

#### TRANSITION BETWEEN TYPES 2 AND 4

*CASE 12—Cardiac hypertrophy, primitive arrangement of chambers and great vessels, patent foramen ovale, left ventricular preponderance, interventricular septal defect, accessory septum in right ventricle which demarcates an aortic chamber, transposition of great vessels, single coronary artery orifice, stenosis of pulmonary artery, fusion of pulmonary valve cusps*

A 1 year old boy had shown marked cyanosis and attacks of dyspnea since birth. Physical examination revealed acute otitis media on the right, marked cardiac enlargement and an inconstant systolic murmur along the sternum. For twelve hours preceding death, the infant remained markedly cyanotic and dyspneic. At autopsy the following additional observations were made: terminal bronchopneumonia, marked pulmonary interstitial emphysema, elongated sigmoid mesentery, large Meckel's diverticulum and general mild visceral congestion. Microscopic examination of the heart revealed marked congestion, edema, many pyknotic nuclei, vacuolation and degeneration of the muscle fibers. The endocardium was found not remarkable.

The heart weighs 90 Gm (normal weight for this age, 44 Gm). It presents a transverse diameter of 7 cm and a vertical diameter of 7 cm, as compared with the transthoracic diameter of 14 cm. The heart resembles a primitive cardiac loop in a striking manner (fig 9). The very large aorta, arching upward and to the left, begins at the extreme right upper portion of the ventricle, so that the right border of the aorta forms a smooth curve with the acute margin of the heart. A very small pulmonary artery takes origin to the left of and slightly behind the aorta. To the left of both vessels are the auricular appendages. The right atrium extends for a distance of 35 mm along the base. Both auricular appendages project forward, side by side, over the base and down the left upper portion of the heart for a distance of 25 mm. The left margin of the left auricular appendage lies directly over the obtuse margin of the heart. Thus the anterior surface presents an oval shape. The left side of the oval is made up of the obtuse margin and the upper margins of both auricles. The right side is composed of

the acute margin and the right border of the aorta. Posteriorly the same relationships hold, the aorta, pulmonary artery, right atrium and left atrium comprising the base from right to left in that order. The largest of the anterior coronary arteries passes downward from the midpoint of the base, which is located in the middle of the right atrium. The largest of the posterior coronary arteries arises from the midpoint of the base between the atria and descends vertically.

The right atrium is very large, measuring 30 mm in transverse diameter and 45 mm in sagittal diameter (including the auricular appendage). It receives the venae cavae and coronary vein in normal positions and shows a slight constriction in the right lateral wall, corresponding to the position of the aorta. The interatrial septum contains a fossa ovalis, measuring 14 by 10 mm, which is partially covered posteriorly by a fenestrated membrane. The three large openings in this membrane have combined diameters of 3 by 9 mm. The right atrio-

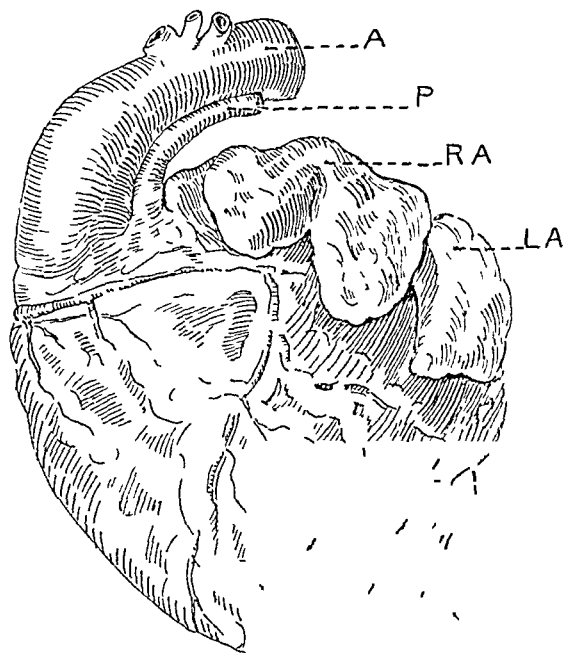


Fig 9 (case 12)—Anterior surface of the heart. *A* indicates the aorta, *P*, the pulmonary artery, *RA*, the right atrium, and *LA*, the left atrium.

ventricular orifice is situated in the floor of the atrium just to the left of and slightly behind the great vessels. It measures 60 mm in circumference and bears three cusps. The left atrium is also very large, measuring 23 mm in transverse diameter and 40 mm in sagittal diameter (including the auricular appendage). It shows the foramen ovale and the openings of the four pulmonary veins in the usual positions. The left atrioventricular opening is immediately to the left of and posterior to the right orifice. It measures 40 mm in circumference and bears normal medioanterior and posterolateral leaflets. The two papillary muscles are normally situated opposite each other on the ventricular walls near the left border of the heart.

The ventricle is divided into right and left portions by an incomplete septum, measuring 15 mm in thickness, which runs in the sagittal plane through a point 2 cm above the apex on the acute margin of the heart. This position, owing to the obliquity of the acute margin, causes the right ventricle to be higher than the

left and makes the septum appear more extensive on the left side. The septum extends up for a distance of 2.5 cm, leaving a round defect superiorly, measuring 15 by 15 mm. This opening is in the posterior superior portion of the septum and lies medially to the medial cusp of the mitral valve. To the right of the defect are the medial and posterior leaflets of the tricuspid valve, while above it is the line of fusion of the atrioventricular rings. The left ventricle is very large, measuring 40 mm in internal vertical diameter and 20 mm in transverse diameter. The walls are 8 mm thick and show the fine trabeculation characteristic of the left ventricle. The mitral valve is at the extreme left. No great vessels issue from that ventricle (fig 10).

The right ventricle is small, measuring 20 by 20 mm (fig 11). It occupies the upper and right portion of the heart and is very heavily trabeculated. The walls

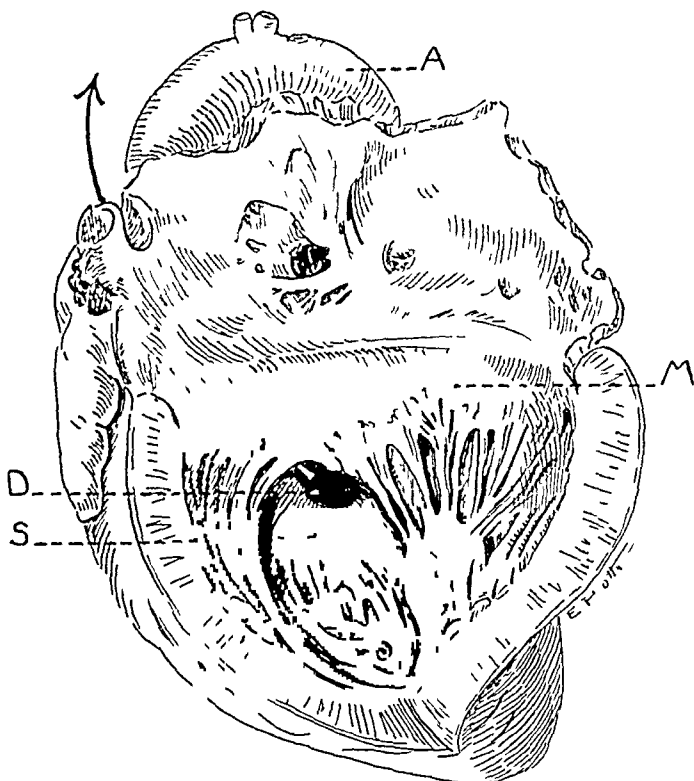


Fig 10 (case 12) —The heart as viewed from the left. The left ventricle and atrium have been opened. *A*, indicates the aorta, *M*, the mitral valve, *S*, the true interventricular septum, and *D*, the interventricular septal defect. The arrow proceeds through the defect into the pulmonary artery.

measure 10 mm in thickness. The interventricular septum, owing to the obliquity of the acute margin, is very much less prominent than it was in the left ventricle, being only 10 mm in height. It shows the defect in the extreme posterior portion of the ventricle. The medial tricuspid cusp is fused to the anterior border of the defect and is seen as an irregular endocardial thickening. The posterior tricuspid leaflet lies to the right of the defect and is partly attached to the anterior inferior border by chordae. The large anterior cusp stretches from the junction of the anterior ventricular wall and the septum to the right posterior portion of the ventricular wall. Both great vessels emerge from the right anterior part of the right



ventricle Between the atretic pulmonary artery and the aorta, parallel to the anterior tricuspid cusp, is a heavy ledge which takes origin near the anterior interventricular septum and courses back and to the right over the ventricular base to insert on the posterolateral wall This ledge measures 1 cm in height and is as prominent as the ventricular septum when viewed from the right side It thus divides the right ventricle into a right, slightly anterior portion, which gives

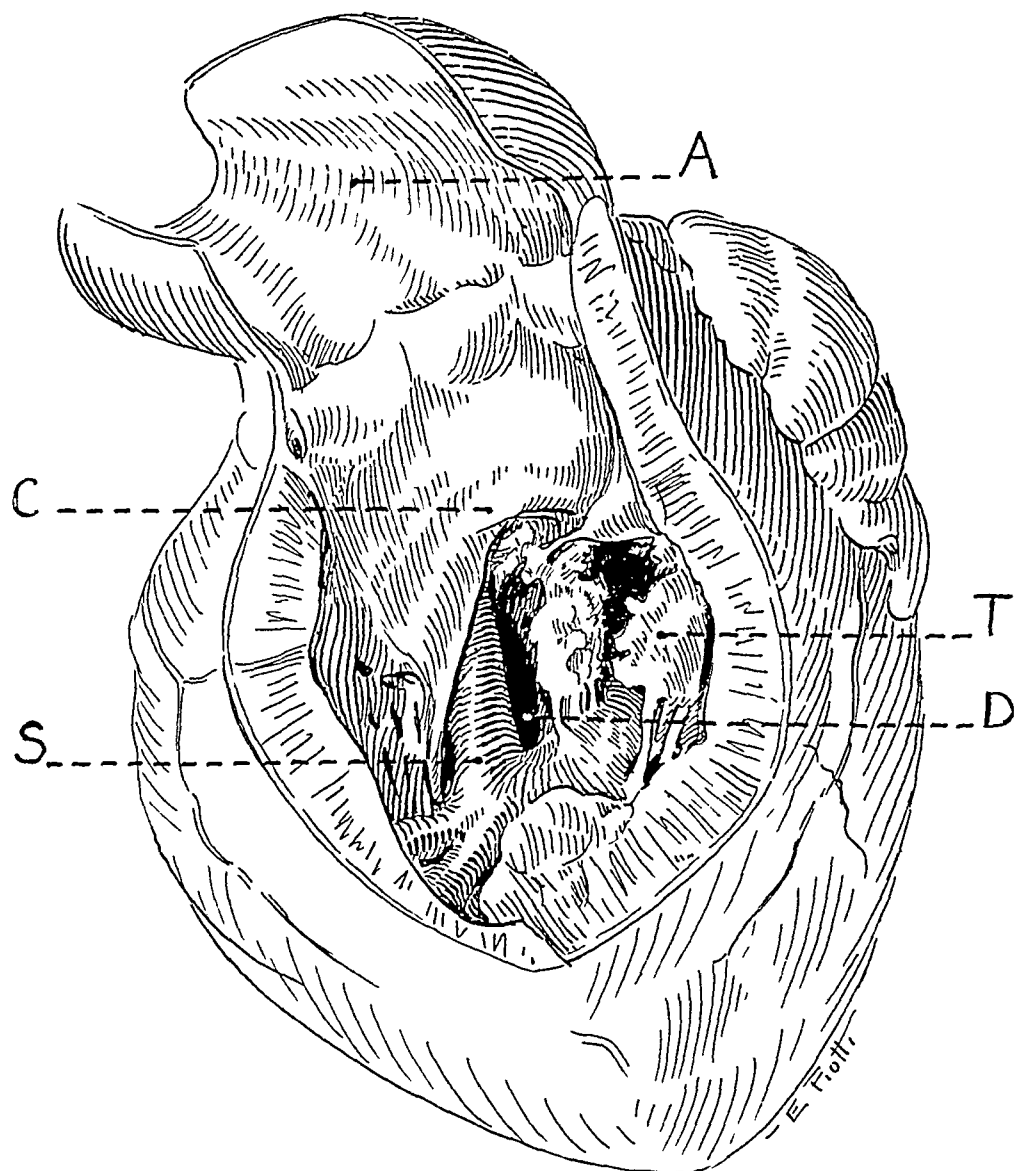


Fig 11 (case 12) —The heart viewed from the right The right ventricle and aorta have been opened *A* indicates the aorta, *C*, the crista supraventricularis, *T*, the tricuspid valve, *S*, the true interventricular septum, and *D*, the interventricular septal defect

rise to the aorta, and a left posterior portion, which contains the atretic pulmonary artery and the tricuspid valve To the posterior border of this ledge is attached the ring of the anterior tricuspid leaflet A small conical papillary muscle is

inserted to the right of the anteromedial portion of the ledge. Its single heavy chorda runs beneath the ledge to insert at the junction of the anterior and posterior cusps.

The aortic valve measures 40 mm in circumference and shows normally formed posterior, right anterior and left anterior cusps. The pulmonary artery measures 12 mm at the valve, but the lumen is closed by the fusion of the tips of the left anterior, posterior and right cusps. The latter two cusps are entirely fused and demarcated only by a ridge in the sinus. The pulmonary artery is attached to the left posterior side of the aorta by fibrous tissue. The right branch of the pulmonary artery passes beneath the aorta. The ductus arteriosus is small, and measures 3 mm in diameter. The branches of the aortic arch are not remarkable.

There is only one coronary orifice. This is situated in the posterior sinus of the aorta. The vessel divides into anterior and posterior branches. The former passes around the aorta to reach the anterior coronary sulcus, where it proceeds from right to left. It gives off the right marginal branch and several twigs which pass down the anterior surface of the right ventricle. None of these resembles the true anterior descending branch. The posterior vessel passes behind the pulmonary artery and then goes from right to left in the posterior coronary sulcus as far as the left border of the heart. It gives off branches which supply the posterior surfaces of the atria and ventricles. The largest branch begins just to the right of the midposterior surface and courses down to the apex directly over the position of the true interventricular septum. Another large branch, given off near the left heart border, passes downward to the left and crosses the mid-point of the obtuse margin. It supplies the lower left anterior portion of the heart.

Such a primitive arrangement as was observed in the structures of this heart has been noted but rarely. Abbott in 1927 cited the cases of Wenner and Birmingham, and Bredt reported 2 cases of his own. Dunner and Kettler have described similar cases. Apparently there has been but little torsion either of the ascending primitive cardiac loop about the descending portion or of the great vessels about one another. In consequence the mitral, tricuspid, pulmonary and aortic valves are placed in that order from left to right in the ventricular cavity. Kettler has given an explanation according to ontogenetic theories which places the cause of the malformation in an insufficient torsion of the cardiac loop so that the bulbous portion fails to migrate to the left and join correctly with the remainder of the heart. The presence of a transposition, with an almost complete loss of the usual spiraling of the great vessels, apparently confirmed Kettler's hypothesis.

In accord with the theory of Spitzer, this transposition would have to be placed as a transitional form between types 2 and 4. The defective true interventricular septum, clearly defined by the course of the posterior coronary artery, lies between the atrioventricular valves to the left of both great vessels. If this had formed the only septum present, a simple transposition of the aorta (type 2) would have resulted. However, the right ventricle is divided by a semicircular ledge which separates the aorta from the pulmonary artery and the tricuspid valve. These relations, together with the assumption that a right ventricular aorta is

present, suggest that the ledge is derived from the anterior tricuspid ledge and the crista supraventricularis, whose normal medial end is now directed to the right because of the marked detorsion of the heart. Were this ledge to hypertrophy and the rudiment of the true septum to vanish, a transposition of the tricuspid valve, in addition to a complete transposition of the great vessels, should result. This will be seen in case 13.

The difference between Spitzer's concept of torsion and that of other writers is most clearly seen in this case. According to the older explanations, the lack of torsion resulted simply and directly, in a geometric fashion, in both the transposition and the failure of the bulbus to migrate to the left of the right atrium. According to Spitzer, the lack of torsion conditions a whole series of mechanically and phylogenetically determined events by the methods previously described—the closure of the left aorta, the reopening of the right aorta, the regression of the true interventricular septum, the hypertrophy of a false septum and the stenosis of the pulmonary artery.

#### TYPE 4 TRANSPOSITION OF THE GREAT VESSELS AND OF THE TRICUSPID VALVE

*CASE 13—Slight cardiac hypertrophy, defective interatrial septum, interventricular septal defects, transposition of great vessels and of tricuspid valve, stenosis of pulmonary artery, transposition of coronary arteries and rotation of their orifices, bacterial endocarditis of pulmonary valve*

A 15 month old girl showed poor appetite, failure to gain, coldness of the extremities and mild cyanosis, intensified by exertion since birth. Development was moderately retarded. She was brought to the hospital at the age of 14 months because of vomiting, slight abdominal distention and pain. Physical examination showed marked cyanosis, irregular respiration, cardiac enlargement (particularly of the left ventricle), blowing systolic murmur and thrill in the tricuspid area, accentuation of the second pulmonic sound and marked clubbing of the fingers and toes. Two weeks before death the child began to have high fever, marked abdominal distention, dyspnea and severe cyanosis. Signs of pneumonia appeared in both lungs. Blood culture showed hemolytic streptococci. A roentgenogram revealed cardiac enlargement, particularly of the left ventricle. The red blood cell count was 7,100,000, the hemoglobin, 100 per cent. At autopsy the following additional observations were made: bronchopneumonia, visceral infarction, acute peritonitis, acute meningitis, abscess of the left lung, congenital maldevelopment of the pyramids of the left kidney. Microscopic examination of the heart revealed bacterial endocarditis of the pulmonary valve, thickening of the tricuspid valve, foci of necrosis and vascular thrombi in the myocardium. It disclosed no evidence of inflammatory change in the tricuspid valve.

The heart is slightly enlarged, and the anterior surface of the ventricles presents a trapezoid whose long diameter is directed to the left and only slightly downward. This abnormality in shape is caused by an abnormal length of the base from the great vessels on the right to the obtuse margin on the left, a short vertical acute margin, a long horizontal obtuse margin, which forms the lower border of the heart, and, last, a large square-shaped prominence at the extreme right, which leads into the aorta. The aorta is situated at the extreme right upper portion of the heart. It is very large and proceeds upward and to the left. Posterior

and to the left of the aorta is the much smaller pulmonary artery, whose course parallels that of the aorta. The extreme right side of the right atrium is obscured by the large aorta, but the left portion, making up the right auricular appendage, appears anteriorly around the left side of the great vessels. To the left of the large right auricle is a much smaller left atrium, whose appendage lies along the left side of the right auricular appendage and marks the left border of the base. Demarcating the prominence of the right side of the anterior surface of the ventricles from the remainder is a shallow sulcus. This sulcus does not contain a coronary artery.

The main portion of the right atrium measures approximately 25 mm in transverse diameter and 26 mm in vertical diameter. The auricular appendage measures 20 mm in transverse diameter and 15 mm in vertical diameter. It is separated from the main portion of the atrium by a shallow constriction caused by the ascending aorta. The walls of the atrium are hypertrophied and heavily trabeculated. The crista terminalis is represented by a heavy ridge in the usual position. The opening of the venae cavae and the coronary vein are normally situated. The eustachian valve is very low, but the thebesian valve is well developed. The interatrial septum is defective. It contains an oval opening, measuring approximately 20 mm in vertical and 10 mm in transverse diameter. The upper and anterior borders of the defect form a low ridge on the corresponding portions of the atrial walls. Posteriorly and inferiorly the margin is crescentic and sharp. On the right side of the interatrial septum is a thin membranous crescentic flap which arises on the superior portion of the wall of the septum and courses 6 mm behind and parallel to the posterior border of the interatrial defect to end by joining with the eustachian valve as it passes in front of the coronary orifice. Between the posterior border of the defect and the flap the endocardium is very thin, and muscle fibers may be seen through it. The right atrioventricular orifice lies at the base of the right atrium with its medial margin adjoining the defective portion of the interatrial septum. The valve ring lies at a 45 degree angle, so that the lateral portion is below the medial portion. The orifice, therefore, is directed downward and to the left. The atrioventricular ring is small, measuring 12 mm in diameter. There are no separate cusps. The valve forms a cone whose orifice at the apex measures approximately 2 mm in diameter. The valve is thickened, and the opening is placed eccentrically, to the right of the center.

The left atrium is small. It shows the large defect in the interatrial septum. The inferior and superior right pulmonary veins unite to form a large pulmonary vein which inserts into the angle between the posterior and septal walls of the left atrium. Two left pulmonary veins open into the atrium in their usual positions. The left atrioventricular orifice is large, measuring 40 mm in circumference. It is situated at the extreme left and posterior portion of the heart. The valve is bicuspid, with the leaflets running obliquely down and to the left. The free edges of the valve are slightly thickened and show several fibrous nodules. The papillary muscles and chordae are not remarkable. The anterior papillary muscle inserts at the left anterior portion of the heart midway between the base and the apex. The posterior muscle is situated directly opposite and close to the anterior group.

The ventricles are separated by a heavy band of muscle tissue, measuring 10 by 10 mm in cross section and 15 mm in length (fig 12). This band stretches from the anterior wall backward and slightly downward in a sagittal plane to reach the posterior wall directly opposite. The right ventricle corresponds to the prominence seen at the right on the anterior surface of the heart. It is only half as large as the left. The lower border of the heavy ledge is concave downward, leaving a

5 by 5 mm defect at the lowermost portion of the ventricles. When viewed from the right side, the upper portion of the ledge presents an anterior and a posterior concavity separated by a small projection between them. The anterior portion bounds the left base of the aorta, which arises from the anterior portion of the small right chamber. The posterior concavity lies directly beneath the right lateral portion of the tricuspid valve, while the projection between the two concavities gives rise to thick chordae and muscular attachments to the base of the midpoint of the anterior part of the tricuspid valve. Below the valve, between it and the posterior concave upper portion of the septum, is an opening into the left ventricle, measuring 7 mm in transverse and 6 mm in vertical diameter. The main portion of the tricuspid valve is situated to the left of this defect, so that the tricuspid valve is largely transposed into the left ventricle. Only the most lateral portion of the tricuspid valve, which is attached to the right portion of the atrioventricular ring, remains in the right ventricle. The aorta arises far anteriorly. Its valve measures 35 mm in circumference, and the cusps are situated in left

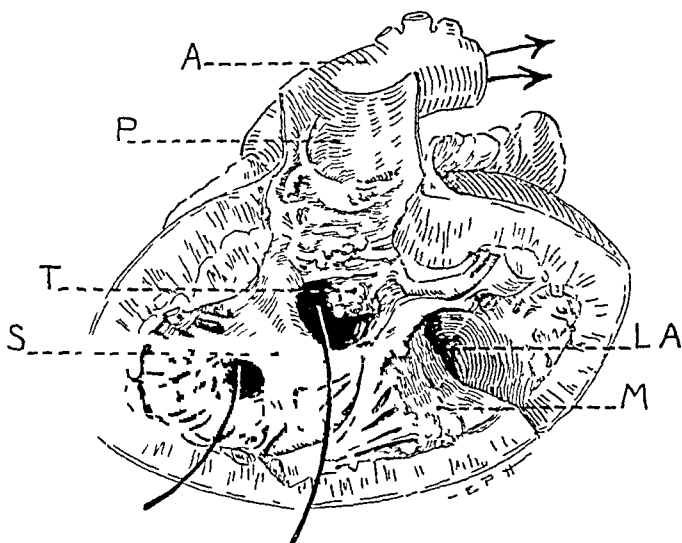


Fig 12 (case 13)—The heart viewed from below and from the left. The left ventricle has been opened and its walls retracted. *A* indicates the aorta, *T*, the tricuspid valve, *S*, the ventricular septum, *LA*, the left atrium, *P*, the pulmonary artery, and *M*, the mitral valve. The arrows proceed through the septal defects, into the right ventricle and out of the aorta.

lateral, anterior and posterior positions. The left and posterior sinuses bear the coronary orifices. A smooth muscular ledge fills the space between the left cusp of the aorta and the anterior upper concavity of the septum. It is directed at right angles to the septum and courses over the right ventricular base from a point below the posterior cusp of the aorta to reach the lateral wall of the right ventricle. There it turns downward and slightly backward to reach the portion of right lateral ventricular wall anterior to the tricuspid valve. The medial edge of this ledge does not quite fill the space between the aorta and the anterior upper concavity of the septum but leaves several small pockets down which a 1 mm probe may be pushed to enter the left ventricle between trabeculations. The left ventricle shows the entrance of the mitral valve far to its left side (fig 12). The pulmonary artery arises from the medial portion just in front of the center of the left ventricle. Its right base is bounded by the anterior portion of the

interventricular septum, while its left side is bounded by a moderately thick ledge which takes origin at the upper anterior part of the septum. The ledge courses in front of the pulmonary artery to reach the left side, where it turns posteriorly across the base and becomes lost in the angle between the mitral and tricuspid valves. The tricuspid valve lies posterior to the pulmonary artery, to which it is partially fused, and to the right of the mitral valve, to whose medial cusp it is also partly fused. The orifice of the tricuspid valve lies in the left ventricle above the large defect in the septum, previously noted. The pulmonary valve is small, measuring 18 mm in circumference. The valve bears right, posterior and left anterior cusps. The right and posterior cusps are fused and are demarcated only by a small ridge in the common sinus. The vegetations of bacterial endocarditis are present on the ventricular surfaces of the leaflets. The vessels arising from the aortic arch and pulmonary artery are normal. The ductus arteriosus is not patent. The coronary artery from the left aortic sinus emerges in front of the pulmonary artery and courses to the left in the coronary sulcus. It gives off several small descending branches to supply portions of the aortic prominence, a large descending coronary artery which is situated anterior to the ledge which bounds the left side of the pulmonary artery in the ventricle. The artery arising from the posterior aortic sinus emerges around the right side of the aorta and proceeds to the left in the posterior coronary sulcus. It gives off a few small branches which supply the right side of the aortic prominence, a large right marginal artery and one large branch passing down and to the left parallel and 15 mm distant from the acute margin. The main artery then goes toward the left border of the heart, near which it gives off a large descending branch which passes down and to the right. This branch occupies a position just to the right of the posterior mitral group of papillary muscles. It meets the branch, previously mentioned, on the posterior surface of the heart, near the right border of the heart.

In the discussion of case 12 it was pointed out that regression of the true interventricular septum and formation of a false septum from the crista supraventricularis and the anterior tricuspid ledge would result in transposition of the tricuspid valve into the left ventricle. Case 13 demonstrates this condition. Only the extreme right lateral portion of the tricuspid valve remains in the right ventricle. The aorta arises from a small chamber separated by a septum from the pulmonary artery and the greater part of the tricuspid valve. The septum therefore corresponds to the false septum in the right ventricle in case 12. However, in case 13 the true interventricular septum has undergone much greater regression. The anterior portion is represented only by the ledge to the left of the pulmonary artery. The location of the posterior part is marked by the position of the descending coronary artery to the right of the mitral papillary muscles, but no rudimentary septum can be seen. The deformed condition of the tricuspid valve and the absence of chordae obscure the details of the structure in that region. Theoretically, the anterior cusp of the valve should span the interventricular defect, since it is derived from the anterior tricuspid ledge. The separation of the most lateral and upper part of the leaflet from the aorta is effected by the bundle running behind the aorta to the lateral ventricular wall. This may be the bulboatrial ledge.

TYPE 5 TRANSPOSITION OF THE GREAT VESSELS WITH  
RUDIMENTARY VENTRICULAR SEPTUM<sup>17</sup>

CASE 14—*Marked cardiac enlargement, functionally intact interatrial septum, single functional ventricle, transposition of great vessels, aortic stenosis, transposition of coronary arteries, rotation of coronary orifices*

A 5½ week old boy was slightly cyanotic for a short period after birth. He entered the hospital at the age of 5 days for the removal of a teratoma of the abdominal wall. The teratoma showed bone, cartilage, voluntary muscle, mucous glands and fibrofatty tissue. The respirations and pulse were unduly rapid during convalescence. Physical examination at that time showed generalized cyanosis, dyspnea, pulmonary rales, cardiac enlargement, accentuated pulmonic second sound and a soft systolic murmur along the right sternal border. Roentgen examination revealed an increase in the size of the heart within two weeks. Severe bronchopneumonia preceded death. At autopsy the following additional observations were made: hemorrhagic bronchopneumonia, visceral congestion, petechial hemorrhages in the basal ganglions and a large umbilical hernia.

The heart is much enlarged, weighing 52 Gm (normal weight for this age, 18 Gm). It is rounded and has no definite apex. The transverse diameter is 6 cm, in contrast to the transthoracic diameter of 10 cm. A small aorta arises from a prominence on the anterior right portion of the ventricles. A much larger pulmonary artery arises posterior to and slightly to the left of the aorta. The heart appears rotated so that the right atrium is placed more anteriorly than usual, being slightly anterior to the left atrium. Both atria and their appendages are large. The right appendage is situated around the right side of the great vessels. The posterior coronary artery descends in a very shallow sulcus from the base down and to the left to meet the obtuse margin slightly above the apex. The anterior descending coronary artery is 0.5 cm from the left border, demarcating a small segment of the left side of the heart. Seen exteriorly, the right ventricle appears to be very much larger than the left.

The right atrium receives the inferior and superior venae cavae and the coronary vein in their normal positions. The thebesian and eustachian valves are well developed. The fossa ovalis measures 10 by 13 mm and is functionally closed by a membrane. A small probe, however, may be passed anteriorly to enter the left atrium. The wall of the atrium is 2 mm thick and is heavily trabeculated. The right atrioventricular orifice measures 62 mm in circumference and bears an anterior, posterior and small medial cusp. The anterior papillary muscle is at the right lateral border of the heart. On the posterior wall, just behind this, is a group of papillary muscles which give rise to chordae to all the cusps.

The left atrium is large, and its walls are hypertrophied, measuring 2 mm in thickness. The orifices of the pulmonary veins enter in their usual positions. The auricular appendage is small, measuring 1 cm in length. The left atrioventricular valve measures 50 mm in circumference and bears medial and lateral cusps. The valve is obliquely situated, running from a right posterior position above to a left anterior position below. There is a large anterior papillary muscle on the left lateral ventricular wall. A small posterior mitral group of papillary muscles is inserted just to the left of the posterior tricuspid papillary muscles. Between

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17 Spitzer (1923) does not include a type 5 transposition. We have added this class in which detorsion appears to be at a maximum. The false septum in this type demarcates a small chamber anteriorly on the right side which gives origin only to the aorta. The case reported by Gerstman is an excellent example.

the two posterior groups is a thick trabecular ledge (*PS*)<sup>18</sup> which runs down on the posterior ventricular wall from the junction of the fused posterior portions of the atrioventricular valves. It is 3 mm wide, 4 mm thick and 1 cm long.

The interventricular septum is not developed. Two heavy ledges course over the base of the common ventricle. The one to the right (*D*)<sup>18</sup> is 15 mm deep and runs from the lower end of the anterior wall just to the right of the midline upward and slightly to the right to reach the base, where it turns laterally behind the aortic opening to be inserted on the right lateral wall just in front of the anterior papillary muscle of the tricuspid valves. It thus demarcates a small chamber, 15 mm deep and 7 mm in diameter, in the right anterior portion of the heart, which corresponds to the prominence noted on the surface. The aorta is the only vessel which arises from this chamber. Posterior and to the left of the anterior portion of the ledge is the pulmonary orifice, which is thus separated from the aorta. On the left side of the pulmonary artery is a smaller ledge (*AS*)<sup>18</sup> which begins in the left anterior wall close to the anterior papillary muscle of the mitral valve. It courses up the anterior wall and then over the ventricular base to the left of the pulmonary orifice. Posterior to the pulmonary artery are the medial and anterior tricuspid leaflets on the right and the anterior mitral cusp on the left. The ledge to the left of the pulmonary artery is situated opposite the large ridge noted on the posterior ventricular wall between the posterior papillary muscles of the mitral and tricuspid valves.

The aorta is narrow, measuring 21 mm in circumference at the valve. The valve contains three cusps—right posterior, left posterior and anterior. The aorta courses upward and to the left, giving off the usual branches from the arch. The orifice above the right posterior cusp gives rise to a right circumflex artery which emerges between the aorta and right atrium, courses in the posterior coronal sulcus and ends in the posterior descending branch after giving off twigs which supply the right posterior surface of the ventricle. The other coronary artery arises above the left posterior cusp, passes in front of the pulmonary artery and divides into an anterior descending and a left circumflex branch which proceeds as far as the posterior sulcus. The pulmonary valve measures 28 mm in circumference and bears left anterior, left posterior and right lateral cusps. The vessel courses up to the left of the aorta and divides. The right pulmonary artery passes beneath the ascending aorta. The ductus arteriosus is represented by a short solid ligament.

*CASE 15—Cardiac enlargement, functionally intact interatrial septum, defective interventricular septum separating an aortic ventricle, complete transposition of great vessels, pulmonary stenosis, transposition of coronary arteries, rotation of aortic coronary cusps, abnormal coronary sinus, patent ductus arteriosus, anomalous band in right atrium.*

A 4 month old girl, one of twins, had shown frequent spells of crying and cyanosis since birth. A peculiar loud cough was noted. Physical examination showed emaciation, mild cyanosis after violent coughing spells, cardiac enlargement and a loud blowing precordial systolic murmur which obscured the heart sounds. A respiratory infection with signs of pneumonia at the bases of both lungs preceded death. At autopsy the following additional observations were made: acute bronchopneumonia and acute early focal necrosis of liver.

The heart is markedly enlarged, weighing 45 Gm (normal weight, 23 Gm). It is approximately oval in shape because of a prominence anteriorly, just to

<sup>18</sup> See discussion, page 489



the right of the midline, which gives rise to a large aorta. The pulmonary artery is only slightly smaller and at its origin is situated in the center of the base of the heart, posterior and to the left of the aorta. Both auricles are approximately equal in size and project forward on either side of the great vessels. A vessel which appears to be the anterior descending branch of the coronary artery begins to the left of the pulmonary artery and runs down, 5 mm from the obtuse margin, to reach the left border of the heart 5 mm above the apex. A second large anterior coronary vessel runs down and to the left from the right upper border of the heart to the acute margin, 1 cm above the apex. The main posterior descending coronary arises from the left third of the base and courses down and to the left to reach the obtuse margin 1 cm above the apex. Thus the main descending coronary arteries demarcate a small segment of the left side of the heart.

The right atrium measures 30 mm in transverse and 20 mm in vertical diameter and has walls 1 mm in thickness. The superior and inferior venae cavae have the usual positions. The latter is bounded by a well marked eustachian valve. Below and to the right is a small imperfect valve which guards a normally situated but very small coronary sinus orifice. The main coronary sinus opening is in an anomalous position in the angle between the inferior vena cava and the interatrial septum. The fossa ovalis measures 7 by 12 mm and is functionally closed by an intact membrane. A small probe may be passed into the left atrium between the limbus and the membrane anteriorly. Just lateral to the opening of the superior vena cava is a muscular cord, measuring 2 mm in diameter and 12 mm in length, which traverses the right atrial cavity from the anterior to the posterior superior wall. Anteriorly the cord joins the crista terminalis in front of the superior vena cava. The right atrioventricular orifice measures 37 mm in circumference and bears a tricuspid valve.

The left atrium receives the four pulmonary veins in their usual position. The left atrioventricular valve is bicuspid and measures 43 mm in circumference.

Only one ventricle is present, measuring 31 mm in transverse and 40 mm in vertical diameter. The walls are 7 to 8 mm thick. There is no division which corresponds to the course of the coronary arteries exteriorly. The tricuspid valve enters on the extreme right side of the common ventricle. The large anterior leaflet is partially divided into medial and lateral portions. It is connected to a group of papillary muscles on the right anterior wall of the ventricle about one-half way between the base and the apex. The posterior papillary muscle is situated behind and to the right on the right lateral wall. Chordae to the small medial and posterior cusps are also given off from the right side of a large group of papillary muscles on the posterior wall of the ventricle slightly to the right of the midline. The left side of this greatly hypertrophied group forms the posterior papillary muscles of the mitral valve. The bicuspid valve is obliquely situated, running from the middle of the posterior wall anteriorly to the left lateral wall of the heart. The cusps are well developed. In the angle between the posterior medial portions of the atrioventricular valves is the orifice of the pulmonary artery, behind which the junction of the medial and medial anterior leaflets of the tricuspid valve is fused with the posterior junction of the mitral cusps. In front of the anterior cusp of the tricuspid valve, thus in front of and to the right of the pulmonary artery, is the base of the aorta, which is bounded by a circle composed of two muscular ledges. The medial ledge (*D*)<sup>18</sup> begins at the anterior wall of the ventricle, to the right of the pulmonary artery, and courses first to the left of the aorta and then posterior to it, where it bends laterally, slightly forward and downward.

to reach the anterior papillary muscle of the tricuspid valve. The ledge lies between the aorta and both the pulmonary artery and the tricuspid valve. It gives off several chordae to the junctions of two parts of the anterior tricuspid leaflet and is connected to the lateral portion of the valve. The remainder of the circle around the base of the aorta is completed by a ridge which runs from the anterior papillary muscle of the tricuspid valve upward and to the left across the anterior wall of the ventricle to reach the starting point of the previously described ridge. A small aortic ventricle, measuring 1 cm in depth, is thus formed anterior to the ledges. The aortic valve is 25 mm in circumference and bears right anterior, left anterior and posterior cusps. Above the left anterior cusp is the opening of a coronary vessel which courses to the left in front of the pulmonary artery to give off the large anterior descending branch and several small descending branches which supply the anterior portion of the ventricle. The anterior descending artery corresponds in position to the space between the pulmonary artery and the medial cusp of the mitral valve. The other coronary artery opens above the posterior aortic cusp. It courses to the right to form a right circumflex artery. This gives off the large right marginal branch, previously described, several small branches which supply the posterior surface, and the large posterior descending coronary artery lying far toward the left border of the heart. The pulmonary artery (situated between the tricuspid valve on the right, the aorta right anteriorly and the mitral valve on the left) measures 26 mm in circumference at the valve, which is guarded by anterior, right and left lateral cusps. The further course of the great vessels is not remarkable. The ductus arteriosus admits a 3 mm probe.

With increasing degrees of detorsion, the plane of the crista supraventricularis would finally pass to the right lateral wall of the right ventricle. In this position detorsion is at a maximum, for the heart has not undergone any torsion at all. The aorta is then placed directly anterior to the pulmonary artery as in the original position in the straight cardiac tube. The crista supraventricularis is rotated to such an extent that it comes in line with the bulboatrial ledge. Hypertrophy of these two structures would result in a septum demarcating an anterior pure aortic ventricle from the remainder of the heart. The septum would separate anteriorly the aorta from the pulmonary artery (role of the crista) and course between the aorta and tricuspid valve posteriorly (role of the bulboatrial ledge). Cases 14 and 15 show the ledge, marked *D*, in this position. The aorta arises from a small chamber in the anterior portion of the right side of the heart. The remainder of the ventricle is a single cavity due to the regression of the true septum ventriculorum. Case 14, however, shows the rudiments of the anterior and posterior true septum quite clearly in the ledge to the left of the pulmonary artery anteriorly (*AS*) and the muscle band between the posterior papillary muscles of the atrioventricular valves posteriorly (*PS*). Moreover, the main descending coronary arteries still mark the site of the true septum. Case 15 demonstrates the aortic ventricle more clearly.

## BULBOVENTRICULAR INVERSION COMBINED WITH TRANSPOSITION

CASE 16—*Cardiac enlargement, patent foramen ovale, interventricular septal defect, closed ductus arteriosus, inversion of ventricles combined with complete transposition of great vessels, stenosis of pulmonary valve, bacterial endocarditis of pulmonary valve and artery, rotation of coronary-bearing aortic sinuses, inversion and transposition of coronary arteries*

A 4½ year old girl was well until four weeks before death. At that time she had a head cold, accompanied by cough, malaise and fatigue. Physical examination showed pallor, an appearance of chronic illness, cardiac enlargement to the left, a sharp snapping accentuated second pulmonic sound, a loud harsh systolic murmur (best heard in the pulmonic area), splenomegaly, hepatomegaly, purpuric areas on the ankles, signs of pneumonia at the base of the right lung and questionably clubbed fingers. There was a gradual downhill course with increasing cardiac decompensation. Ascites, peripheral edema and hydrothorax appeared. The electrocardiogram was suggestive of myocardial disease, showing a diphasic T wave in lead I and slight slurring of the QRS complex. At autopsy the following additional observations were made: terminal hemolytic streptococcus bacteremia, vegetative endocarditis caused by a gram-positive diphtheroid bacillus, acute mediastinitis, multiple pulmonary infarcts, visceral congestion, healing pulmonary tuberculosis in the left lung and peripheral edema. The heart on microscopic examination showed occasional areas of fibrosis. There were no other abnormalities except for the areas of bacterial endocarditis.

The heart is enlarged and unusually triangular in appearance, because of a prominence at the left upper portion of the anterior surface. The large aorta arises from this prominence. The pulmonary artery lies posterior and to the right of the aorta. The long axis of the heart is directed downward and to the left. The right atrium is large and projects to a great extent around the right side of the great vessels. The left atrium lies behind the great vessels. The anterior descending coronary artery courses down and to the left from the space between the aorta and the pulmonary artery. The posterior descending branch passes down and to the left, corresponding in position to the right margin of the interventricular septum. Both the aorta and the pulmonary artery course directly upward.

The right atrium shows the usual venous openings, but the valves of the inferior vena cava and coronary vein are rudimentary. The fossa ovalis measures 14 by 10 mm and is closed by a membrane except for a 3 by 7 mm opening anteriorly. The right atrioventricular orifice measures 70 mm in circumference and bears two large cusps situated anteromedially and posterolaterally. The free edges show numerous verrucous and granular vegetations. Two large papillary muscles are present, situated on the anterior and posterior walls of the right ventricle, opposite one another. Thus the right atrioventricular orifice has the cusps and papillary configuration of a mitral valve.

The right side of the interventricular septum is smooth. In its upper midportion it shows a defect, measuring 12 by 8 mm, which is surrounded by heavy muscle tissue save posteriorly, where the medial portions of the atrioventricular valves are fused with each other. The pulmonary artery takes origin from the midportion of the right ventricle, between the anterior leaf of the right atrioventricular valve and the interventricular defect. The pulmonary valve is small (31 mm in circumference) and bears right posterior, left posterior and anterior cusps. The left posterior and anterior cusps are fused and are demarcated only by a ridge in the common sinus. The right posterior cusp bears the same intimate relation to the anterior leaflet of the right atrioventricular valve as the left posterior

cusps of the aorta does normally to the aortic leaflet of the mitral valve. The pulmonary valve, artery and branches show the coarse vegetations of bacterial endocarditis.

The left atrium is slightly smaller than the right. It receives the four pulmonary veins as usual and shows the small opening of the foramen ovale. At the base of the atrium is the left atrioventricular orifice, measuring 54 mm in circumference. This valve bears anterolateral, posterior and medial cusps. The anterior papillary muscle is situated at the left anteroinferior portion of the left ventricle and gives chordae to the anterior and posterior leaflets. The posterior papillary muscle, attached to the posterior and medial leaflets, is directly behind and very close to the anterior group. The medial cusp is attached by numerous chordae to the inferior aspect of the interventricular defect. The left atrioventricular valve and papillary muscles correspond, therefore, to those normally present in the right ventricle. In the upper posterior portion of the interventricular septum is the defect which opens into the most posterior portion of the right ventricle. The aorta arises from the extreme anterior portion of the left ventricle, from the prominence seen on the anterior surface. The valve measures 51 mm in circumference and has normal-appearing posterior, right anterior and left anterior cusps. A heavy ledge, measuring 10 mm in width and 5 mm in depth, begins near the anterior papillary muscle of the left atrioventricular valve. It courses to the interventricular septum and then passes up the left side of the septum to the junction of the posterior and right cusps of the aortic valve. This ledge resembles those designated by the letter *T*<sup>16</sup> in the previous cases. A smaller ledge courses over the base of the left ventricular cavity between the aorta and the anterior tricuspid leaflet. It passes medially and posteriorly to form part of the arched upper muscular border of the interventricular septal defect. In form and position it resembles the ledges previously designated by the letter *A* (bulboatrial ledge).

The aorta does not have the usual intimate relationship to the left atrioventricular orifice but is separated from it by a muscular space 1 cm in width.

The interior of the right ventricle measures 25 mm in transverse and 45 mm in vertical diameter, while the left measures 25 by 40 mm. The cavity on the left is very heavily and coarsely trabeculated, resembling the usual right ventricular structure. The right ventricle has the fine trabeculation in this heart.

The aorta gives off the usual branches. The ductus arteriosus is represented by a ligamentous cord. The coronary orifices are situated above the posterior and right anterior cusps. The artery from above the former divides almost at once to form a few branches, which pass around anterior to the aorta and supply the left ventricle, and a left circumflex branch which goes from left to right in the posterior coronary sulcus to end in the posterior descending artery. The coronary artery arising above the right anterior cusp gives off a branch which passes around the right side of the aorta in front of the pulmonary artery to form the anterior descending artery. The artery then continues as the right circumflex and gives off a right marginal branch and several smaller vessels, supplying the posterior surface of the right ventricle.

*CASE 17—Cardiac enlargement, large patent foramen ovale, widely patent ductus arteriosus, inversion of the ventricles combined with transposition of great vessels, abnormal septum separating left aortic ventricle, atresia of left atrioventricular orifice, anomalous origin of transposed and inverted coronary arteries, coarctation of aorta.*

An 11 day old boy had shown failure to nurse properly, choking spells and difficulty in breathing since birth. Physical examination showed deep cyanosis,

marked dyspnea, marked enlargement of the heart, rough precordial systolic murmur heard loudest at the apex and a gallop rhythm. Roentgenograms revealed cardiac enlargement and congestive peribronchial mottling. At autopsy the following additional observations were made: bronchopneumonia, central necrosis of the liver, passive congestion of the viscera and visceral hemorrhages. The histologic examination of the heart disclosed nothing remarkable.

The heart weighs 38 Gm (normal weight, 18 Gm). The shape is somewhat triangular because of a small prominence at the left upper region and the great length of the base to the right of the great vessels. The apex points down and slightly to the left. The small aorta arises from the extreme left anterior portion of the base and the pulmonary artery, equal in size to the aorta, lies behind and to the right of it at the origin. The base of the ventricles to the right of both vessels is surmounted by a large right atrium. The left atrium, situated behind the great vessels, is very small. The anterior interventricular groove begins at the base to the right of both aorta and pulmonary artery and traverses down and to the left to the apex. The posterior groove passes down from left to right, in contrast to the usual direction, and ends to the right of the apex on the acute margin. Both great vessels course directly upward, parallel to each other.

The right atrium measures 30 by 20 mm and receives the venae cavae and coronary vein in normal position. The venous valves are well developed. The crista terminalis is thick, and the auricular appendage is large, hypertrophied and heavily trabeculated. The fossa ovalis measures 10 by 14 mm and is half closed in its posterior and inferior portions by a thin cribriform membrane. This leaves a patent foramen ovale, measuring 7 by 10 mm. The right atrioventricular orifice measures 35 mm in circumference and bears only two large leaflets, situated anteromedially and posterolaterally. An anterior papillary muscle group is present on the right anterior wall of the ventricle, while a posterior group is situated opposite but somewhat more to the right on the posterior wall. The right atrioventricular valve thus has the characteristics of a mitral valve.

The left atrium measures but 20 by 15 mm. It receives the openings of the pulmonary veins in the usual position and shows the patent foramen ovale as previously described. A left atrioventricular orifice is lacking, but in the floor of the atrium is a small depression corresponding to an atretic valve.

The ventricles are unequally divided by a small septum which separates the small left anterior prominence, noted previously on the anterior surface of the heart, from the remainder of the ventricular cavity (fig 13). The right ventricle measures 35 by 25 mm, in contrast to 15 by 10 mm for the left. The only entrance to the small left ventricle is through a defect, measuring 3 mm, in the posterosuperior portion of the septum. The upper margin of the defect is formed by a thick band of muscle tissue which separates the left anterior base of the pulmonary artery from the aorta. The aorta is the only vessel to arise from the left ventricle. Its valve measures 16 mm in circumference and bears anterior, left posterior and right posterior cusps. The right and left posterior cusps are fused and are demarcated only by a ridge in the common sinus. The aorta after giving rise to the innominate, left common carotid and left subclavian arteries in that order narrows rapidly. The circumference just before the entrance of the ductus arteriosus is 3 mm. The degree of coarctation is marked, and the ductus seems to form the descending aorta. Distal to the ductus arteriosus, the aorta measures 15 mm in circumference.

The pulmonary artery arises just to the left of the center of the heart. To the right is the medial cusp of the right atrioventricular valve, which is fused to the right side of the base of the artery. A heavy ledge, measuring 4 mm in thickness and 20 mm in length, courses down the posterior wall of the ventricle from the right posterior base of the pulmonary artery to end just to the left of the posterior papillary muscle of the right atrioventricular valve. Superiorly this ledge is adjacent to the upper portion of the interventricular septum. Joining the left base of the pulmonary artery to the upper portion of the septum is a thin narrow membrane which furnishes the base of the depression in the floor of the left atrium. It therefore represents the atretic left atrioventricular valve—located in the small space between the ledge previously described, the interventricular septum and the left posterior base of the pulmonary artery. The pulmonary valve measures 20 mm in circumference and bears posterior, left anterior and right anterior cusps. The artery courses directly upward, and the left branch passes under the ascending aorta. The ductus arteriosus measures 10 mm in circumference and is the main source of the blood supply to the descending aorta.

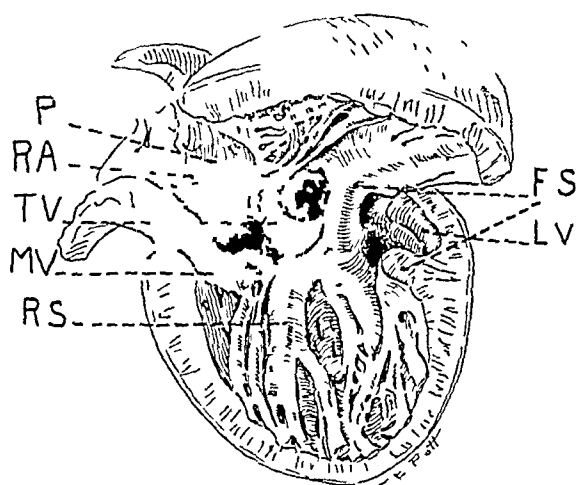


Fig 13 (case 17)—The heart viewed from in front. The anterior wall of the ventricles has been retracted upward. *P* indicates the pulmonary artery, *RA*, the right atrium, *TV*, the site of the atretic left (tricuspid) atrioventricular valve, *MV*, the right (bicuspid) atrioventricular valve, *RS*, the rudimentary "true" interventricular septum, *FS*, the "false" interventricular septum, and *LV*, the left ventricle.

The coronary arteries arise from the left posterior and right posterior aortic sinuses. The former vessel turns around the left border of the heart and then passes from left to right in the posterior coronary sulcus. It gives off few small branches to supply the aortic prominence, a large posterior descending coronary artery and several small branches which supply the posterior surface of the ventricles to the right of the posterior interventricular sulcus. Two vessels open into the right posterior aortic sinus. The anterior artery emerges between the aorta and the pulmonary artery to form the anterior descending coronary artery. The posterior vessel passes in front of the pulmonary artery from left to right in the anterior coronary sulcus, then around the right margin of the heart to course from right to left in the posterior coronary sulcus. It gives

off descending branches to the anterior surface of the right ventricle, a large right marginal branch and several vessels which course down the right posterior ventricular surface

Two examples of corrected transposition (cases 16 and 17) have been added to complete the present series. The first shows normal situs solitus of the body and the atria. The right ventricle, however, bears a bicuspid valve, while the left has the tricuspid valve. Furthermore, the left ventricle is heavily trabeculated, while the right has the usual fine trabeculation of the normal left ventricle. This suggests an inversion of the bulboventricular loop. Nevertheless, a true mirror picture should have the aorta in right posterior and the pulmonary artery in left anterior position. In case 16 the position of the vessels forms a mirror picture not of the normal heart but of a heart in type 3 transposition. The corrected transposition in this case therefore must consist of partial inversion coupled with a type 3 transposition. The explanations of the various ledges and of the position of the coronary arteries are similar to those in the usual crossed transposition. It is interesting to note the neutralizing effect of the two serious anomalies of partial inversion and transposition in regard to cardiac function and viability. Unfortunately, the pulmonary artery is still subject to the same stenosis and valvular deformities, incident to transposition, which may provide the *locus minoris resistentiae* for bacterial endocarditis.

The ventricles and great vessels in case 17 form a mirror picture of a type which has not as yet been discussed in this presentation. If the crista supraventricularis were to come into line with the tricuspid valve, one would expect hypertrophy of both anterior and posterior tricuspid ledges. A further stage of the process would be obliteration of the tricuspid orifice exactly as the pulmonary artery is occasionally obliterated between hypertrophied ledges in type 2 transposition. The false interventricular septum in this event would demarcate a small anterior ventricle which gives rise only to the aorta (as in type 4). The rudiment of the true interventricular septum should be present on the posterior wall of the ventricle medial to the false septum.

Case 17 is an example of such a transition between types 3 and 4.<sup>19</sup> It shows the atretic valve in the expected position between the remnant of the true interventricular septum, the pulmonary valve and the false septum. In addition, however, an inversion of the bulboventricular loop has occurred. The bicuspid valve is on the right, while the atretic (probably tricuspid) valve is on the left. The aortic ventricle is on the extreme left side of the heart instead of on the right (see cases 13, 14 and 15). The position of the coronary arteries tends to bear out these deductions as to the origin of the anomalies (see the following paragraph).

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<sup>19</sup> A similar case in situs solitus is that reported by Smetana and analyzed by Spitzer.

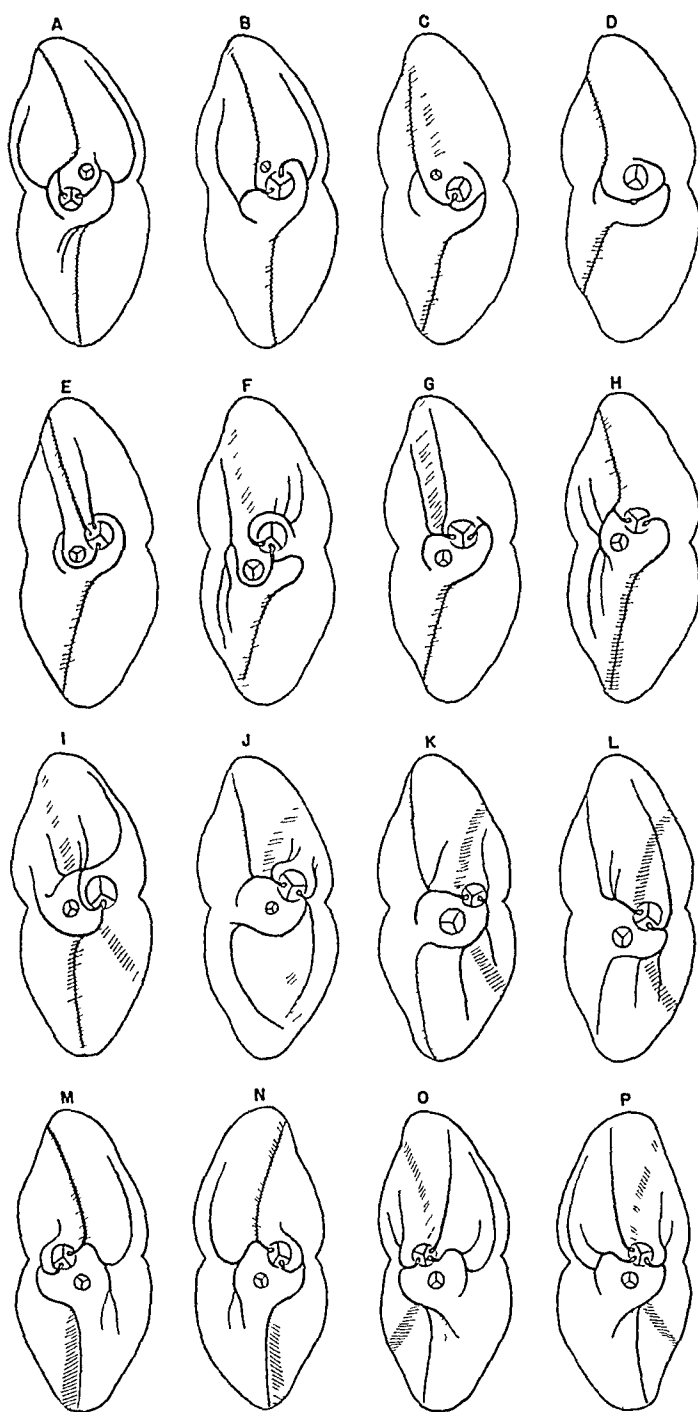


Fig 14—The course of the coronary arteries in the various cases. The heart is depicted as though it were sectioned coronally from the apex to the base. The anterior surface is above, and the posterior surface is below. The view is from above. The striped shaded areas denote the septums present. The stippled areas represent the position of the rudimentary portions of the true septums. *A* represents the normal heart, *B*, case 4, *C*, case 6, *D*, case 7, *E*, case 8, *F*, case 9, *G*, case 10, *H*, case 11, *I*, case 12, *J*, case 13, *K*, case 14, *L*, case 15, *M*, case 16, *N*, a mirror image of case 16, *O*, case 17, and *P*, a mirror image of case 17.



## CORONARY ARTERIES

An examination of the coronary arteries in this series reveals two interesting facts (fig 14) As Spitzer has pointed out, an increasing transposition of the vessels should be expected as the bulbus, so to speak, rotates in a counterclockwise direction This is demonstrated very well by cases 8, 9, 10 and 11 In case 8 the course and origin of the vessels are essentially normal The detorsion of the bulbus, however, has increased the path of the anterior descending artery, since the latter must pass behind the pulmonary artery Case 9 shows transposition of part of the right circumflex and the posterior descending artery, while in case 10 the process has extended to the left circumflex and the anterior descending arteries The latter take origin from the left posterior aortic sinus, which was originally in the right anterior position In case 11 the process has been completed, all branches of the left coronary artery now arise from the original right coronary orifice

The relations of the anterior and posterior descending arteries to the ventricular septum in these cases give support to Spitzer's hypothesis The anterior descending coronary arteries in cases 8 and 9 are situated to the left of the actual interventricular septum The logical conclusion is that it still marks the location of the true anterior septum even though that structure has disappeared In case 6 the coronary artery marks the left, or true septal, side of the composite septum present Cases 8 and 10 show a new anterior descending coronary artery which has apparently developed in response to the circulatory demands of the false septum Nevertheless the original anterior artery, although transposed to the original right aortic sinus in case 10, still marks the position of the absent true interventricular septum Cases 13, 14 and 15 demonstrate the same condition in regard to the posterior septum which is postulated as false in these cases In the 3 cases, both anterior and posterior arteries mark the vanished or rudimentary true interventricular septums and not the false septums which demarcate the aortic ventricle

It will be noted that the mirror pictures of the coronary vessels in cases 16 and 17 closely resemble the actual course of the arteries in the similar types of transposition in situs solitus (cases 11 and 14, respectively) This fact lends support to the belief that transposition of the great vessels and of coronary arteries in these cases has the same pathogenesis as that in situs solitus and that the condition of partial inversion does not affect this mechanism

## FINAL COMMENT

Spitzer's main contribution is a theory of normal cardiac development The fundamental postulate of that theory is the orderly development of the organ, as a unit, in response to the varying conditions, forces and demands in a series rising from fishes to birds and mammals

It admits of no fortuitous variations which disregard the phylogenetic interrelations of these groups. Furthermore, the theory is capable of explaining on mechanical grounds some of the short cuts in the ontogenetic recapitulation of these events. For these reasons Spitzer's theory is unique and assumes importance even though the secondary application to teratology may not invariably meet with success.

Criticisms have been leveled, for the most part, not at the theory of normal cardiac development but at its application to cardiac malformations. Teratology, however, is only one of the many modes of attack. But few facts of cardiac embryology or phylogeny which are capable of testing the theory have been advanced. Pernkopf and Wirtinger, as has been mentioned, advanced an entirely different view of cardiac development. They stated, in contrast to Spitzer's theory, that the septum arises as a spiraled structure, that dynamic and phylogenetic factors are of no importance in the "crossing over" of the pulmonic and systemic circuits, that the cardiac tube is fixed at the openings of the pulmonary veins and not peripheral to them and that the crista supraventricularis arises from the leaf of the bulboatrial ledge on that side which contains the ventral great vessel. They cannot find evidence of a reptilian stage in human development or of any homology between the reptilian right aorta and the niche designated by Spitzer in human hearts. On the other hand, Fuchs, in a study of the development of the heart of *Vanellus cristatus*, was able to support Spitzer's views concerning the formation of the ventricles, the importance of the proximal bulbar swellings *a* and *b* in the formation of the ventricular septum, the migration of the aortopulmonary septum from bulbar swelling II to III, the derivation of the crista supraventricularis from the aortopulmonary septum and the homology of the trabecula septomarginalis to the *Muskelleiste* of Greil.

The greatest amount of criticism has been directed against the complexity and apparent improbability of Spitzer's explanations of the malformations. For example, in type 3 transposition, the entire architecture of the heart may appear normal except for the interchanging of the aorta and the pulmonary artery. Sato and Kung even demonstrated a normal course of the atrioventricular bundles in this type of case. One hesitates to accept the opinion that the ventricular septum is any other than the normal one, or that the right leaf of a long-vanished bulboatrial ledge forms an apparently normal crista supraventricularis for the aorta in the right ventricle. The changes postulated by Spitzer seem too radical ever to construct a cardiac architecture which conforms to the normal so closely as it does in this type of case. Yet it is possible, as has been attempted in this presentation, to arrange anomalous hearts in a logical series leading up to the type in question and to explain both

the individual cases and the transitions in accord with theoretic expectations. Furthermore, Spitzer showed in 1920 that a structure may appear in the position of the normal crista supraventricularis which must be derived from some other anlage, probably the bulboatrial ledge.

It has been stated that the relatively normal course of the atrioventricular bundles in the ordinary type of transposition is proof against the presence of a false interventricular septum. However, since all types of transposition except types 4 and 5 have normal posterior septums, the positions of the atrioventricular node and crus commune should not be disturbed. Furthermore, the right bundle normally follows the septal course of the crista supraventricularis down the true septum.<sup>20</sup> This position should be disturbed but little when the crista takes over the function of the true anterior septum. Normal variations in the terminal branches are so great as to vitiate any conclusions from their course in abnormal hearts. In types 4 and 5 the atrioventricular bundles should show an abnormal course. No studies of this type have been reported.

Another objection is that no case has yet been reported in which two definite aortas were present. Even though this type would be very rare on theoretic grounds, it would be necessary to have one in order to complete the series of cases.

More serious criticisms concern Spitzer's assumption that the heart must develop as a whole. Thus the actual portion of the heart may be approximately normal in cases of transposition in which the postulated detorsion should affect the venous as well as the arterial ends of the primitive loop. The great frequency of interatrial defects, however, tends to bear out the theory. In other cases it may be that the detorsion is taken up by a change in the angle between the ventricles and atria as shown by the angle between the planes of the interatrial and ventricular septums in case 5.

It is impossible to meet the objection that the heart cannot develop as a whole since isolated inversions of the bulboventricular loop with a counterclockwise spiral at the arterial end may be associated with another counterclockwise spiral at the venous end of the primitive loop. In the present state of knowledge, however, it is equally impossible to conceive of the pathogenesis of any partial inversion. Any attempt to do so on the basis of tendencies inherent in the germ plasma is confronted with the experiments of Stohr, previously mentioned.<sup>21</sup> Any explana-

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20 Walmsley, in Quain's "Elements of Anatomy," p. 81. See bibliography, under "General References."

21 Theories of normal cardiac development which are based solely on an inherent spiraling of the septums have to meet a similar objection (e.g. the theory of Pernkopf and Wirtinger).

tion on the only other ground of environmental forces, such as those of the blood stream, has to face the problem of how these factors can exert opposing mirror picture effects on adjacent, functionally interdependent portions of a complex organ such as the heart

#### SUMMARY

In a review of the hypotheses concerning the pathogenesis of transposition of the great cardiac vessels, particular attention has been paid to the theories of Rokitansky and of Spitzer, since they represent the ontogenetic and phylogenetic aspects of this malformation. An attempt has been made to present in detail the essence of the theory of Spitzer as gathered from a study of all pertinent literature. Nineteen examples of transposition from the Infants' Hospital and the Children's Hospital collection have been analyzed in illustration of that theory. This series included examples of overriding aorta, complete transposition of the great vessels, transposition of the tricuspid valve, bulboventricular inversion, atresia of the tricuspid orifice and cor biatriatum pseudotriloculare with mitral atresia. One case showed a very rare primitive arrangement of the chambers of the heart, while another showed the combination of fetal endocarditis and aortic transposition. Incidental malformations of the heart included various deformities of the interatrial and interventricular septums, valvular abnormalities, aortic coarctation and a complete vascular circle around the trachea and esophagus.

In each case, particular attention was paid to the course of the main coronary arteries. Inversions, corrected transposition and the relation of mitral atresia to transposition were briefly examined.

It may be concluded that the theory of Spitzer marks an important advance in the understanding of cardiac development and certain cardiac malformations. Although many data, accumulated from embryology, phylogeny and teratology, tend to support the theory, further proof will be necessary before it can be accepted completely.

This paper represents a companion study to a series of reports to be published elsewhere by Dr. Paul Emerson and Dr. Hyman Green, who, with Dr. Kenneth D. Blackfan, have stimulated the study of congenital heart disease for the past twenty years at the Children's Hospital in Boston.

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# VITAMIN C DEFICIENCY AND INTESTINAL FUSOSPIROCHETOSIS

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The importance of fusospirochetal organisms in the production of infections within the intestinal tract has been of interest since Koch<sup>1</sup> described fusospirochetal intestinal infections in fatal cases of scurvy. Zimserling<sup>2</sup> reported lesions in the intestines of patients who had severe fusospirochetal infections of the mouth, pharynx and lungs. His observations were made during the period of partial starvation in Russia between 1916 and 1920. Bouty,<sup>3</sup> McNeill<sup>4</sup> and Broughton<sup>5</sup> also cited instances of primary fusospirochetal infection of the intestinal tract. Ariel<sup>6</sup> reported autopsies in which spirochetes and fusiform bacilli were found within the intestinal wall in 1 of 5 cases of tuberculous intestinal ulcers, in 3 of 6 cases of typhoid ulcers and in a single instance of bacillary dysentery.

Streicher and Kaplan<sup>7</sup> found spirochetes predominating in 3 of their 65 cases of ulcerative colitis. Goadby<sup>8</sup> saw fusiform bacilli in cases of ulcerative colitis. Muhlens,<sup>9</sup> Langendorfer and Peters<sup>10</sup> found spirochetes in chronic ulcerations of the colon. Smith<sup>11</sup> stated, "Patients having a prolonged convalescence from typhoid fever, bacillary dysentery,

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1 Koch, W. Veroffentl. a. d. Geb. d. Kriegs- u. Konstitutionspath. **2** 1, 1920

2 Zimserling, W. D. Veroffentl. a. d. Geb. d. Kriegs- u. Konstitutionspath. **5** 78, 1928

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8 Goadby, K. Lancet **1** 959, 1916

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10 Langendorfer, J. W., and Peters. Munchen med. Wchnschr. **58** 12, 1921

11 Smith, D. T. Oral Spirochetes and Related Organisms in Fusospirochetal Disease, Baltimore, Williams & Wilkins Company, 1932



typhus fever, cholera or other intestinal diseases, especially amoebic dysentery, should be suspected of having a fusospirochetel infection of the intestines”

These observations were supported by the experimental work of De Jemal and Achitou,<sup>12</sup> who found a great increase of cecal spirochetes in guinea pigs after they were placed on a scurvy-producing diet. In 1933 McConkey and Smith<sup>13</sup> showed that an adequate supply of vitamin C usually protects guinea pigs against intestinal tuberculosis. Vitamin C deficiency predisposed their animals to the development of ulcerative tuberculous intestinal lesions. Smith<sup>11</sup> stated that 3 of 5 guinea pigs which had been kept on a diet partially deficient in vitamin C and also fed 0.5 cc of fusospirochetel pus which came from a spontaneous subcutaneous abscess in another guinea pig showed essentially normal intestinal tracts. The other 2 had numerous deep necrotic ulcers in their ceca. Smears from these ulcers showed large numbers of fusiform bacilli, spirochetes and cocci.

With these observations in mind it was thought that it would be of interest to see how often fusospirochetes could be found in intestinal lesions and also to attempt to produce the lesions experimentally.

#### AUTOPSY STUDIES

The autopsy protocols of the department of pathology on all instances of diphtheritic and ulcerative colitis, typhoid fever, and bacillary dysentery (Flexner) and enteritis due to *Giardia* and *Ascaris* were studied. There were 24 such instances. Sections of the intestines were examined for fusiform bacilli. These were found in both the mucosa and the submucosa in 12 cases. In 2 of the 12 cases Levaditi stains were available, and in both cases spirochetes were observed in the intestinal wall. In all but 2 of the 12 cases some other disease was present which could cause a break in the mucosa.

The clinical histories were examined to learn whether any conditions were present which might favor fusospirochetel infections. It was found that all the patients had been on diets which were deficient in some important nutritional elements, but the most constant history was that of a general deficiency of vitamins, particularly of vitamin C.

Some observers believe that one of the most common physical evidences of vitamin C deficiency is severe pyorrhoea alveolaris. Almost without exception our patients who were past the second decade of life had this lesion, associated with a considerable amount of gum infection and abscesses.

#### EXPERIMENTAL MATERIALS AND METHODS

Only male guinea pigs were used, in order to eliminate possible complications associated with pregnancy. The animals used in experiment 1 were young guinea pigs, weighing between 170 and 280 Gm. In experiment 2 only adult guinea pigs, weighing between 573 and 850 Gm, were used.

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<sup>12</sup> De Jemal and Achitou, cited by Smith<sup>11</sup>

<sup>13</sup> McConkey, M., and Smith, D. T. J. Exper. Med. **58** 503, 1933

The mixtures of fusospirochetal organisms were obtained by scraping the margins of the gums of patients who showed various degrees of untreated pyorrhoea alveolaris. The debris obtained was mixed with physiologic solution of sodium chloride to form a thick suspension. This suspension, in amounts varying from 0.2 to 0.5 cc, was injected through a 26 gage needle into the dorsal subcutaneous tissue of the hindlegs of normal guinea pigs. The subcutaneous tissue afforded a medium with reduced oxygen tension, in which the fusospirochetal organisms grew rapidly, producing a large necrotic abscess within two to four days. The fusospirochetal organisms were aspirated from these abscesses and fed to the experimental animals. Dark field examination of these mixtures demonstrated 40 to 60 actively motile spirochetes in each oil immersion field. The site of aspiration was closed with collodion, to allow the abscess to refill, thus affording opportunity for repeated withdrawals of pus.

The greatest difficulty was encountered in producing sufficient quantities of the fusospirochetal material to keep the average daily feeding at a high level. If the dose inoculated into the subcutaneous tissue was too large, the infection either produced a large necrotic slough at the site of injection or spread rapidly along the fascial planes, thus causing death within twenty-four to forty-eight hours.

In addition to the virulence of the organisms, which was markedly increased as they were passed from animal to animal, the age and physical condition of the inoculated animal also influenced the size of the optimal injection dose. It is, therefore, impossible to specify a definite amount to be injected. A trial and error procedure proved satisfactory when the animals were carefully observed. The amounts given in this experiment varied from 0.1 to 2 cc.

Although the mixture of fusospirochetal organisms was foul, no difficulty was experienced in feeding it to the guinea pigs. They ate it from the end of a 2 cc syringe with great relish. The amount taken at a single feeding varied between 0.1 and 2 cc.

The basic diet consisted of hay and some autoclaved whole milk mixed with rolled oats and salt. In experiment 2 the basic diet was at times supplemented with brewers' yeast, in an effort to increase the animals' consumption of the food. This diet was given in unlimited amounts. Cod liver oil and tomato juice were fed directly into the animals' mouths.

When the animals were moribund, they were killed with inhalation ether, and autopsy was performed immediately. During autopsy the intestine was kept moist with physiologic solution of sodium chloride until placed in the fixing fluid. The upper and lower ends of the intestine were tied, and the intermediate portion was removed intact. The cecum was then detached and incised along the lesser curvature, exposing its mucosa. Following this, the fecal contents were removed by gentle washing with physiologic solution of sodium chloride, and the mucosa was thus exposed for inspection and fixation.

The cecum was placed in solution of formaldehyde U S P diluted 1:10. The small intestine was fixed as follows. The same fixative was gently forced through its lumen, and while the intestine was slightly distended, each end was tied and then the whole was immersed in the fixative. The large intestine was fixed in a similar manner.

After the tissues had fixed for several days, sections were taken from the small intestine and cecum. The area to be sectioned was determined by examining the specimen for possible lesions and taking sections from all areas suggestive of ulceration or hemorrhage. All tissues were embedded in paraffin. Those to be used for general study were stained with hematoxylin and eosin, those to be

examined for bacteria were stained according to MacCallum's method, those to be studied for iron were stained by the potassium ferrocyanide method, and those to be searched for spirochetes were prepared by the method of Levaditi

#### EXPERIMENT 1

This experiment was made in order that we might study the effect of a vitamin C-deficient diet and fusospirochetes on young guinea pigs. The animals were divided into four groups as follows

1 The guinea pigs received the basic deficient diet supplemented by 0.3 cc of cod liver oil daily. After clinical signs of vitamin C deficiency developed, they were fed varying amounts of the fusospirochetal material

2 The guinea pigs received the basic deficient diet supplemented by 0.3 cc of cod liver oil and 10 cc of tomato juice daily. These animals, receiving a non-deficient diet, were fed fusospirochetal organisms in amounts similar to those given to group 1

3 The guinea pigs received the basic deficient diet supplemented by 0.3 cc of cod liver oil and 10 cc of tomato juice daily. They were not given the fusospirochetal organisms

4 The guinea pigs received the basic deficient diet supplemented by 0.3 cc of cod liver oil daily

Each experiment lasted eight weeks

*Group 1—Animals Fed a Vitamin C-Deficient Diet and Organisms*—There were 14 guinea pigs in this group. The animals retained their appetites until they became moribund. The first sign of deficiency was a decrease in activity, followed within a few days by a loose, watery foul diarrhea and loss of weight, which became so serious that it was necessary to give them a few cubic centimeters of tomato juice occasionally. In this way they were kept alive for two to five weeks after the development of a definite scorbutic condition of the joints. The gums, however, never showed any lesions

The mixture of organisms was fed to these guinea pigs soon after signs of deficiency developed. From that time until their deaths they were given an average daily dose ranging between 0.05 and 0.07 cc. The total amounts given were between 0.5 and 1.5 cc

*Morbid Anatomy* The knee joints of these animals were stiff and hemorrhagic. The ceca of 5 guinea pigs each contained several definite, though small, hemorrhagic areas in the mucosa. No ulcers were found, but in 1 cecum a small area was seen which suggested beginning ulceration. In 1 cecum the hemorrhages were so prominent that they could be seen before the cecum was opened. Each cecum was abnormally thin. Sections of the intestines showed large amounts of iron in the phagocytes of the submucosa. MacCallum and Levaditi stains showed no organisms below the mucosal surface

*Group 2—Control Animals Fed Organisms*—There were 6 guinea pigs in this group. These guinea pigs displayed no ill effects and gained weight throughout the experiment. They were fed fusospirochetal pus during the same time as the animals of group 1. The average daily dose received ranged between 0.5 and 1.05 cc

*Morbid Anatomy* There were no changes in the knee joints. The wall of the cecum in every instance was thick and in no case presented evidence of hemorrhage or ulceration. Some of the sections of the intestines showed a few

phagocytes in the submucosa. There was no interruption of the mucosa, and no organisms were found below the mucosal surface in either the bacterial or the Levaditi preparations.

*Group 3—Control Animals Without Organisms*—There were 4 guinea pigs in this group. They always appeared well.

*Morbid Anatomy* There were no stiff joints. In the ceca there were no hemorrhages nor was there prominence of lymphoid tissue. Some of the phagocytes of the submucosa of the intestines contained slight amounts of blood pigment. There were no interruptions in the mucosa. Bacterial and Levaditi preparations showed no organisms below the mucosal surface.

*Group 4—Vitamin C-Deficient Animals Without Organisms*—There were 4 guinea pigs in this group. They were kept alive by occasionally giving them a few cubic centimeters of tomato juice. Scorbutic lesions developed, equal in extent to those found in the animals of group 1.

*Morbid Anatomy* The knee joints were hemorrhagic and stiff, with little swelling. Every cecum was thin and contained areas of hemorrhage sometimes as large as 1 cm. in diameter. Sections of the intestines showed little iron in the phagocytes of the submucosa. There were no interruptions in the mucosa, and bacterial and Levaditi sections revealed no organisms below the mucosal surface.

## EXPERIMENT 2

This experiment was similar to experiment 1 except that adult guinea pigs were used.

*Group 1—Animals Deficient in Vitamin C to Which Organisms Were Given*—There were 12 guinea pigs in this group. Five of the animals did not adjust themselves to the diet and died before any evidence of scorbutic lesions developed. The remaining 7 animals were much more difficult to regulate on the scurvy-producing diet than were the young guinea pigs in experiment 1. Their appetites varied considerably from day to day, and they required more attention than the young animals. The first evidence of deficiency of vitamin C in the adult animals was a profuse watery foul-smelling and usually bloody diarrhea accompanied by loss of weight. Although there was more swelling in the joints of the older animals, the joint motion was not as limited as in the young guinea pigs. Petechiae were noted in the gums a few days after the onset of symptoms of involvement of the joints. The teeth became loose but did not fall out.

As soon as evidence of deficiency was noted, these guinea pigs were started on the feedings of fusospirochetal pus. The average daily dose ranged between 0.09 and 0.4 cc. The total amounts given ranged from 1.2 to 10 cc. These animals died from two to six weeks after the feedings were started.

*Morbid Anatomy* The knee joints were hemorrhagic, swollen and stiff. The teeth were loose, and the gums contained hemorrhages. The ceca were thin, and in several of them hemorrhagic areas were seen. In 1 cecum there was a punched-out ulceration measuring 6 mm. in diameter. Sections of the intestines contained moderate to large amounts of iron in the phagocytes of the submucosa. The cecum mentioned as containing a large punched-out ulcer presented microscopically an ulcerated area including the mucosa and submucosa. This ulceration was found to contain numerous fusiform bacilli, spirochetes, cocci and other bacilli. None of the remaining sections contained interrupted mucosa or organisms below the mucosal surface.

*Group 2—Control Animals Fed Organisms*—There were 4 guinea pigs in this group. These animals continued to grow and gain weight. They were given feedings of organisms during the same period as the animals in group 1. The average daily feeding ranged between 0.22 and 0.35 cc.

*Morbid Anatomy* The ceca showed no hemorrhages or ulcerations and were thick walled. No iron was found in the phagocytes of the submucosa of the intestines on microscopic examination. The mucosa was intact, and no organisms were demonstrable below the mucosal surface.

*Group 3—Control Animals Without Organisms*—There were 3 guinea pigs in this group. These animals were never sick, and they gained weight throughout the experiment.

*Morbid Anatomy* The ceca contained no hemorrhages or ulcerations. Microscopically, a small amount of iron was found in the phagocytes of the intestinal submucosa. There were no interruptions in the mucosa, and no organisms were demonstrable.

*Group 4—Animals Deficient in Vitamin C Without Organisms*—There were 3 guinea pigs in this group. They all presented stiff joints, and their diet had to be supplemented with some tomato juice to keep them alive.

*Morbid Anatomy* The knee joints were swollen, hemorrhagic and stiff. The ceca were thin and contained small areas of hemorrhage without ulceration.

It is interesting to compare the lesions of the alimentary tract produced by vitamin C deficiency in the young animal as observed in experiment 1 with the lesions in the adult guinea pig. The location and the severity of the hemorrhages were not the same. In the young animal the gums were not affected, whereas the gums of the adult guinea pig were involved early. Although there was considerable hemorrhage in the cecum of the young guinea pig, bloody diarrhea never developed, but in the adult animal it did.

Microscopically, the phagocytes of the submucosa contained a moderate amount of iron. There were no areas of ulceration, and no organisms could be demonstrated below the mucosal surface.

#### COMMENT

A microscopic study of sections of intestines obtained at autopsy in 24 cases of intestinal disease, including diphtheritic and ulcerative colitis, typhoid fever, dysentery due to Flexner's bacillus and enteritis due to *Giardia* and *Ascaris*, revealed fusiform bacilli in 12 instances. In only 2 of these instances were Levaditi stains available, and in these spirochetes were seen. It is interesting that in all but 2 of the 12 cases in which fusiform bacilli were found infections were noted which could have caused a break in the intestinal mucosa. All of these patients had been on a diet deficient in vitamin C. This type of diet, as shown by McConkey and Smith,<sup>13</sup> lowers the resistance of the intestines to tuberculous infection and, we believe, also to other infections.

The manner in which the resistance of the intestines to infection is lowered is probably complex, but our experiments suggest one way in which this may occur. The experimental animals on a vitamin C-deficient diet showed numerous small hemorrhages in the intestines. Our experimental evidence indicates that at times these hemorrhages are

sufficiently large to cause necrosis and thus a break in the mucosa which allows the organisms present to gain a foothold. It is thought that it was in this manner that the one ulcer in our experiments and the two reported by Smith<sup>11</sup> occurred.

What part this mechanism played in the clinical cases is difficult to evaluate. The evidence of other etiologic agents at autopsy does not preclude the possibility that there may have been some agent other than the vitamin C deficiency before the fusospirochetal infection started. The work of Koch,<sup>1</sup> however, indicates that extreme vitamin C deficiency leads to definite intestinal lesions with fusospirochetes present.

From a survey of the literature and of our own work it appears that when there is a break in the mucosa of the intestines caused by parasites, bacteria or a deficiency of vitamin C the fusospirochetal organisms, if present, may cause further damage. At this time there is, however, no indication that the intestines can be damaged by fusospirochetes without the aid of some other factor.

#### SUMMARY

In 24 instances of intestinal disease of varied types the intestinal lesions found at autopsy were examined microscopically. In 12 of these the lesions showed fusiform bacilli. The 12 patients had all been on vitamin C-deficient diets, and all but 2 had infections which could cause breaks in the mucosa.

Of 21 guinea pigs fed on a vitamin C-deficient diet and also given fusospirochetes, 1 acquired an intestinal lesion, while none of the control animals showed such a lesion.

It is suggested that fusospirochetes can gain a foothold in the intestine when there is a break in the mucosa. In our studies such breaks occurred as a result of hemorrhages in the mucosa from vitamin C deficiency as well as through the activity of bacteria and protozoa.

# ORGANIC LESIONS PRODUCED BY POLYVINYL ALCOHOL IN RATS AND RABBITS

## A TOXICOPATHOLOGIC INVESTIGATION OF AN EXPERIMENTAL THESAURISIS

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NEW YORK

Polymerized vinyl alcohol (polyvinyl alcohol  $[\text{CH}_2 = \text{CHOH}]_n$ ) is one of several plastic substances developed during recent years by the chemical industry. The commercial, impure product is used in the manufacture of resins, lacquers and other products, while the pure polyvinyl alcohol is employed in the production of synthetic surgical threads of absorbable and nonabsorbable types and as a substitute for gelatin in foodstuffs (Braun<sup>1</sup>, Herrmann and Haehnel<sup>2</sup>). Polyvinyl alcohol consists physicochemically of large molecular aggregates of the unsaturated vinyl alcohol molecules. The molecular size, as well as the physicochemical qualities (solubility, viscosity, color and other properties), of the polymerized compound, depends on the method of production and on the subsequent treatment of the finished product. Polyvinyl alcohol is a white, tasteless and odorless powder. It is soluble in water, glycol and glycerin and insoluble in ethyl alcohol, acetone and many other organic solvents (xylene, chlorinated hydrocarbons and others). It can be precipitated from an aqueous solution as white floccules by the addition of various salts (sodium thiosulfate, ammonium sulfate, sodium sulfate and others) and ethyl alcohol. The resulting fluffy precipitate of hydrated polyvinyl alcohol floats on the surface. When compressed it exhibits a rubber-like elasticity, which, however, gradually decreases on drying, leaving a white crumbling matter. This precipitated product is less soluble in water than the original compound. The colloidal aqueous solutions of polyvinyl alcohol are viscous liquids at room temperature and form soft gels when placed in a refrigerator. The firmness of these gels depends directly on the concentration of the polyvinyl alcohol. Polyvinyl alcohol can be used as a protective colloid in the preparation of colloidal solutions of metals (gold, silver, chromium hydroxide, iron

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From the Warner Institute for Therapeutic Research

1 Braun, R. Ueber das Verhalten des Polyvinylalkohols im tierischen Stoffwechsel und das morphologische Verhalten des Gewebes gegenüber Faden aus Polyvinylalkohol, Inaug. Dissert., Leipzig, 1936

2 Herrmann, W. O., and Haehnel, W. Ber. d. deutsch. chem. Gesellsch. 60 1658, 127

hydroxide and others) As aqueous solutions of polyvinyl alcohol do not become infected with bacteria, they may possess distinct advantages over gelatin solutions for certain purposes

Polyvinyl alcohol gives some of the reactions characteristic for carbohydrates (test of Carletti and the reaction of Fleig and Ihl) and in several other respects behaves similarly to certain large molecular carbohydrates, such as starch A concentrated solution of polyvinyl alcohol assumes a blue-violet color on the addition of a solution of iodine in potassium iodine (compound solution of iodine U S P [Lugol's solution]) The blue color fades on heating and returns on cooling Polyvinyl alcohol combines with formaldehyde, just as starch does, but the product formed, in contrast to the formaldehyde compound of starch, is insoluble in water and does not swell (in water) (Herrmann and Haehnel<sup>2</sup>, Staudinger, Frey and Starck<sup>3</sup>)

Little information exists regarding the biologic effects produced by this interesting substance Herrmann and Haehnel<sup>2</sup> mentioned briefly that they did not observe any untoward effects in several generations of mice receiving polyvinyl alcohol in their dry food or with their drinking water Braun,<sup>1</sup> who investigated the biologic qualities of polyvinyl alcohol more thoroughly, reported that polyvinyl alcohol threads (marketed under the name "Synthofil R" surgical threads) elicited in the tissue a local reaction characterized by the appearance of multinucleated foreign body giant cells Neither the oral nor the subcutaneous introduction of the polyvinyl alcohol into rabbits and dogs was followed by local or systemic organic lesions Braun observed, however, that polyvinyl alcohol administered subcutaneously was in part excreted through the kidneys and was demonstrable in the urine The various unusual physicochemical properties of this substance suggested the desirability of a more extensive experimental investigation of its biologic qualities, especially as such studies may give some additional information on the physicochemical action mechanism of certain biologically important large-molecular substances or aggregates (proteins, polysaccharides, lipids) under normal and under pathologic conditions (immunity reactions, malignant tumors, storage diseases and other conditions)

#### EXPERIMENTAL OBSERVATIONS

The polyvinyl alcohol used in the following experiments was of the commercial type<sup>4</sup> The substance was administered in an aqueous solution sub-

3 Staudinger, H, Frey, K, and Starck, W Ber d deutsch chem Gesellsch 60 1782, 1927

4 The polyvinyl alcohol was obtained from the R & H Chemicals Department of E I duPont de Nemours & Company



cutaneously to albino rats and intravenously to rabbits. It was fed also in a powdered form, mixed with the stock diet, to albino rats.

*Subcutaneous Introduction*—Twelve albino rats, 2 months old, weighing from 70 to 88 Gm each, received a subcutaneous injection of 1 cc of a 5 per cent aqueous solution of polyvinyl alcohol five times per week. One rat died after fifteen injections, while the others survived a course of twenty treatments. At the end of the experimental period (four weeks) 6 rats were killed for a pathologic study of the immediate effects of the treatment. The five survivors were killed two weeks later for the determination of any late effects. The rats were in good health during the entire experimental period and gained weight continuously during this period. At the time of death they weighed from 113 to 194 Gm.

The rat which died spontaneously showed at autopsy an appreciably enlarged, firm, grayish red spleen and shrunken kidneys with a coarsely granular, peculiarly greenish yellow-brown surface. The subcutaneous tissue at the site of injection was swollen and had a grayish jelly-like texture. The postmortem examination of the other 11 rats of this series revealed similar changes in the subcutaneous tissue. The tissue was, however, somewhat firmer and more yellowish white. The spleens were enlarged moderately and were dark red and firm. The kidneys of 2 rats showed greenish brown granular changes of the cortex. The other organs grossly were normal.

The microscopic examination of the sections stained with hematoxylin-eosin showed the following changes:

*Skin* The epidermis and appendages were normal. The subcutaneous connective and muscle tissues were infiltrated diffusely by a homogeneous or finely granular grayish blue-stained material, which was situated in some instances in sinusoidal cavities or had been taken up by numerous balloon-like distended histiocytes possessing a foamy cytoplasm. There were occasional smaller and larger groups of multinucleated giant cells, surrounding a somewhat deeper blue-stained matter and containing in their cytoplasm highly reflective stringy inclusions (fig 1). Smaller and larger necroses, surrounded by lymphocytes and leukocytes, were scattered throughout the subcutaneous tissue.

*Lungs* The lungs of most of the rats were normal. Several of them showed, however, a more or less diffuse mononuclear and lymphoid infiltration of the interstitial tissue and of the interalveolar septums. In a few of the peribronchial and peribronchiolar infiltrations of this kind a considerable admixture of eosinophilic leukocytes was noted. In addition to these nonspecific chronic inflammatory reactions, there occurred in the lungs of several rats focal accumulations of histiocytes of a foam cell character, containing in the cytoplasm a finely granular grayish blue pale matter. The endothelial lining of several small blood vessels was swollen and proliferated, and some of the endothelial cells revealed foamy cytoplasm. The lumens of such vessels were filled by a homogeneous grayish blue matter. In a few instances capillary vessels in the interalveolar septums evidently were blocked entirely by the development of small granulomas consisting of mononuclear cells and multinucleated giant cells surrounding a grayish blue central homogeneous mass (fig 2).

*Mediastinal Lymph Nodes* Small groups of balloon-like, swollen reticular cells containing a finely granular bluish gray matter were present in the pulp.

*Heart* The myocardium was normal in all rats. There were, however, 2 rats in which small accumulations of large mononuclear foam cells were scattered in the interstitial tissue.

*Thymus* This organ was normal.

*Aorta* The vessel was normal.

**Liver** In all rats there was an increase as well as a swelling of the Kupffer cells. Where this alteration had taken place in mild to moderate degree, small solid nodular accumulations of these cells were met occasionally, scattered throughout the normal hepatic parenchyma. In some rats, however, the activation and swelling of the Kupffer cells were very diffuse and marked and this change was

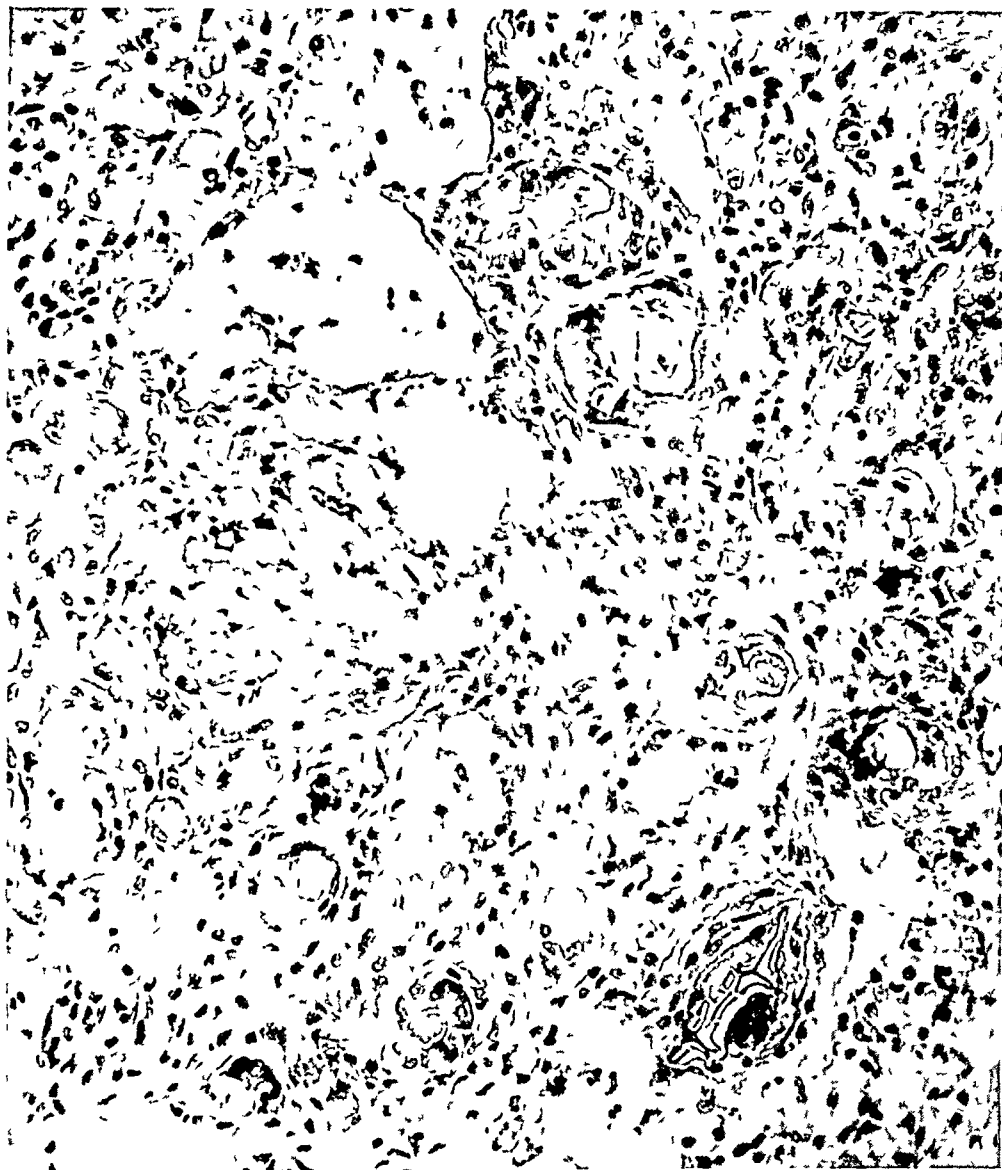


Fig 1—Subcutaneous tissue showing diffuse imbibition with polyvinyl alcohol and presence of mononuclear foam cells and multinucleated giant cells, the latter containing highly reflective stringy inclusions of modified polyvinyl alcohol

associated with the presence of numerous smaller and larger solid reticulo-endothelial nodules located mainly near or around small vessels. The nodules were in general well circumscribed and round but were sometimes of an elongated, bandlike shape or were interconnected by broader cellular branches. Often the cells composing these nodules were arranged in a whirl-like fashion. Three different types of cells were found in these nodules. The majority of the cells

were oval shaped, rather small and provided with similarly shaped, moderately sized, dark staining nuclei. They were often the only kind present with the exception of a minor admixture of eosinophilic leukocytes found in some of the nodules. An appreciable number of nodules also contained large pale-stained cells with large round nuclei and often a cytoplasm showing a foamy structure.

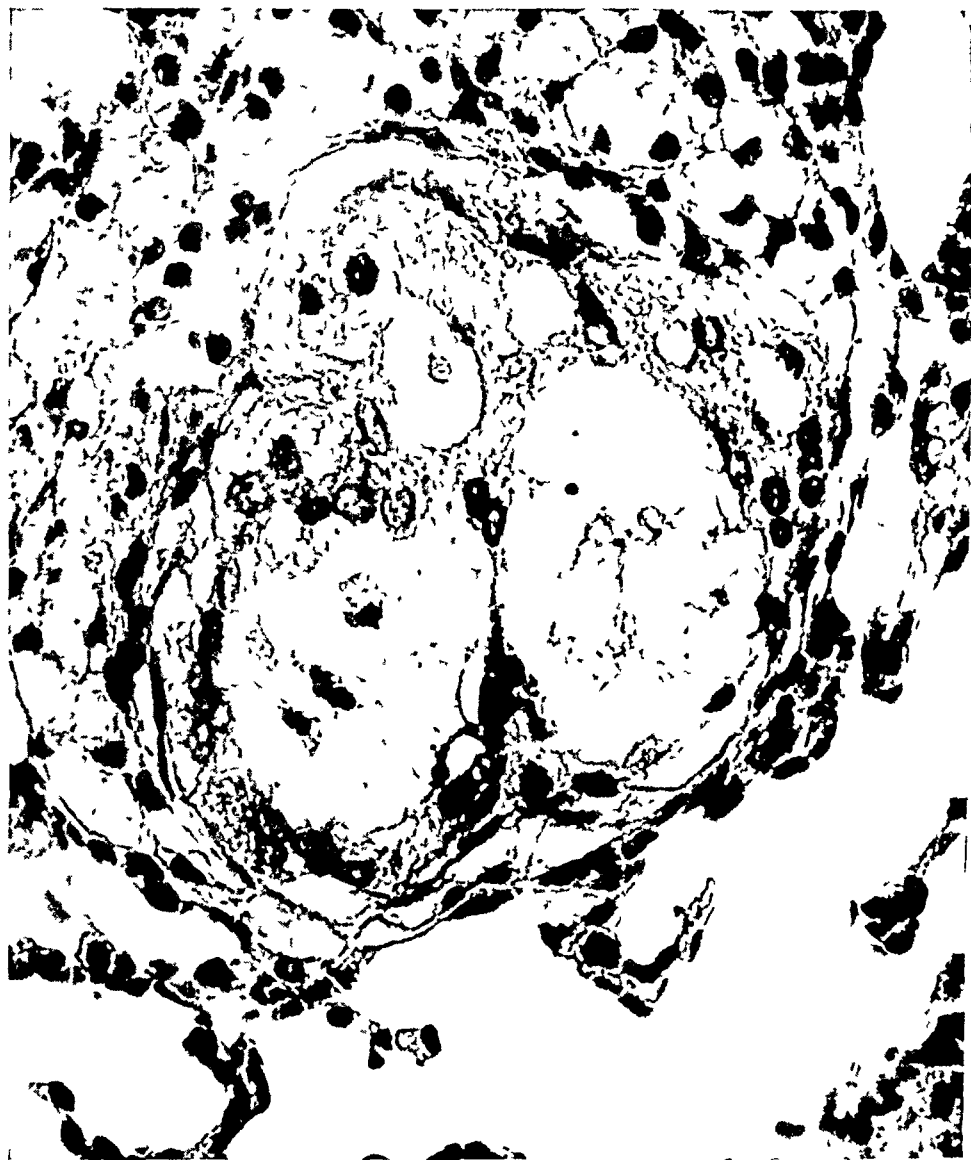


Fig 2—Distended capillary of the lung filled with polyvinyl alcohol, which is surrounded by mononuclear foam cells and proliferated and swollen endothelial cells.

The third cellular type observed in these nodules was represented by multinucleated giant cells or syncytial masses without distinct cellular outlines. The nuclei were arranged in wreath or crescent formation, and often the cytoplasm contained large vacuoles or was foamy. These cells surrounded not infrequently a small central bluish-stained homogeneous mass. Pale blue globules occasionally were seen also within the cytoplasm of isolated, markedly swollen Kupffer

cells. Small groups of balloon-shaped histiocytes with foamy cytoplasm were very rarely in the periportal connective tissue, which showed more often a mild to moderate eosinophilic infiltration. The liver cells were normal in the great majority of rats. Animals with marked reticuloendothelial changes revealed moderate pericentral vacuolar degeneration, while small hyaline necroses were found in 1 rat.

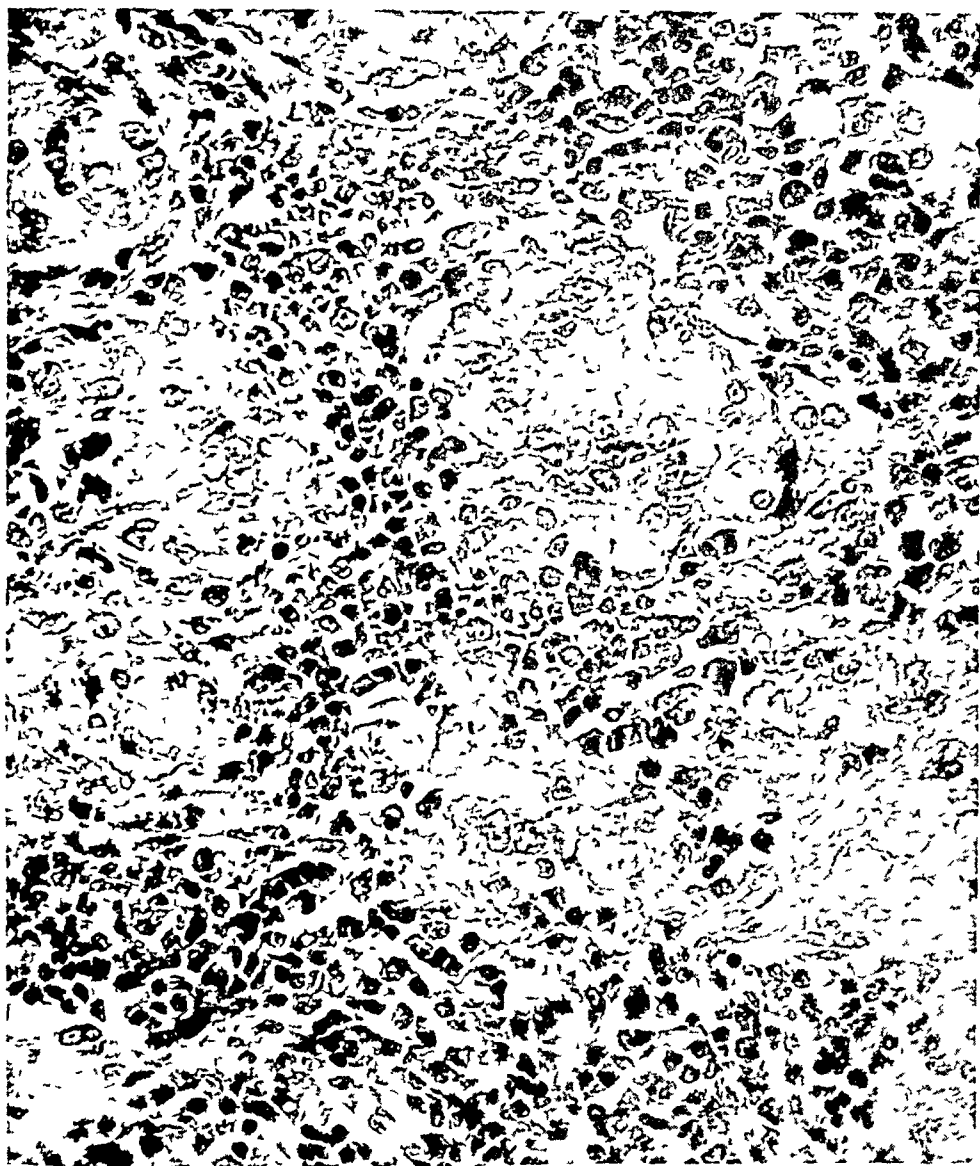


Fig 3—Splenic pulp with swollen reticular cells of foam cell type. Multi-nucleated giant cells are numerous.

**Spleen.** The sinusoidal endothelium and the reticulum cells were increased in number and swollen in all animals. These changes reached extreme degrees in several animals. The entire pulp seemed to consist of a solid mass of large pale-stained cells, which sometimes were arranged in nodules similar to epithelioid cell tubercles, a resemblance made still closer by the frequency with which multi-nucleated giant cells were found within these nodular arrangements (fig 3). The

cytoplasm of the swollen reticuloendothelial cells as well as of the foreign body giant cells was often vacuolated or foamy. In general, the follicles were well preserved.

**Abdominal Lymph Nodes** The great majority of lymph nodes examined were normal. Some of them, however, showed small nests of foam cells and occasionally also multinucleated giant cells within the area of the pulp.

**Adrenals** These organs were normal in most of the rats. Some showed swollen and vacuolated reticuloendothelial cells in the medullary zone. In 1 rat the medulla contained a small solid focus of proliferated reticuloendothelial cells, arranged in a whirl-like fashion.

**Kidneys** The great majority of the kidneys showed a more or less extensive granular hyaline degeneration of the tubular epithelium. In some instances this process had led to complete hyaline necrosis of the entire epithelial lining of some tubules in the cortical zone. In some of the rats these were the only tubular changes present. Others, however, showed additional extensive and rather unique tubular lesions. The tubular lumens were filled in several instances by casts, leukocytes and desquamated swollen epithelial cells with foamy cytoplasm. This foamy type of tubular degeneration was marked especially in the corticomedullary zone. Balloon-like, distended cells often were lining the tubules. In other tubules the epithelial lining apparently had become defective, and multinucleated giant cells formed an inner coating of the former tubules (fig 4). Complete absence of any epithelial lining was encountered not infrequently. This condition usually was associated with considerable cystic distention of the lumens, resulting in the production of a spongy structure in some parts of the kidneys.

In those instances in which there was little pathologic change in the renal parenchyma the interstitial connective tissue was essentially normal. In some instances in which moderate parenchymatous lesions were present, there was a mild to moderate mononuclear infiltration, involving mainly wedge-shaped areas of the cortex and being composed of foam-cellular histiocytes. Small groups of multinucleated giant cells were seen occasionally. In regions with markedly degenerated tubular epithelium the interstitial tissue showed sometimes marked imbibition of a faintly blue-stained homogeneous substance, presenting thereby a myxomatoid appearance. Wherever this imbibition had reached extreme degrees, the original tissue seemed to have broken down, leaving smaller and larger cysts filled with the homogeneous matter and surrounded by a lattice work of preserved basement membranes. A late outcome of these lesions was apparently present in the kidneys of 1 rat, in which considerable thickening and fibrosis of the interstitial tissue, similar to that found in chronic nephrocirrhosis, were seen in addition to marked tubular and glomerular lesions.

The most constant pathologic change observed in the glomeruli was the ballooning of one, several or many endothelial cells in the capillary tufts. This endothelial vesiculation apparently was preceded by swelling and proliferation of the affected cells, which during the preparatory stage showed a foamy cytoplasmic structure. At this time the nuclei were arranged sometimes in irregular clusters. With the further progress of this alteration the appearance became more cystic, the intracellular cavities being filled with a finely granular matter staining grayish blue (fig 5). The ultimate result of this development was complete disintegration of the endothelium, leaving a grapelike bunch of cysts, the walls of which were formed by the preserved and often hyalinized swollen fibrillar elements of the tufts. The reduction in number of cells and the generalized hyalinization of the tufts were followed by atrophy and shrinkage. The marked swelling of the

glomeruli caused in some instances close contact with the thickened and cellular wall of Bowman's capsules and resulted in cellular adhesions between these two parts

Homogeneous small blue globules were found occasionally within the capillary lumens of the glomerular tufts—in those of the afferent vessels in the pedicle—as well as in those of capillaries located in the interstitial tissue of the cortex

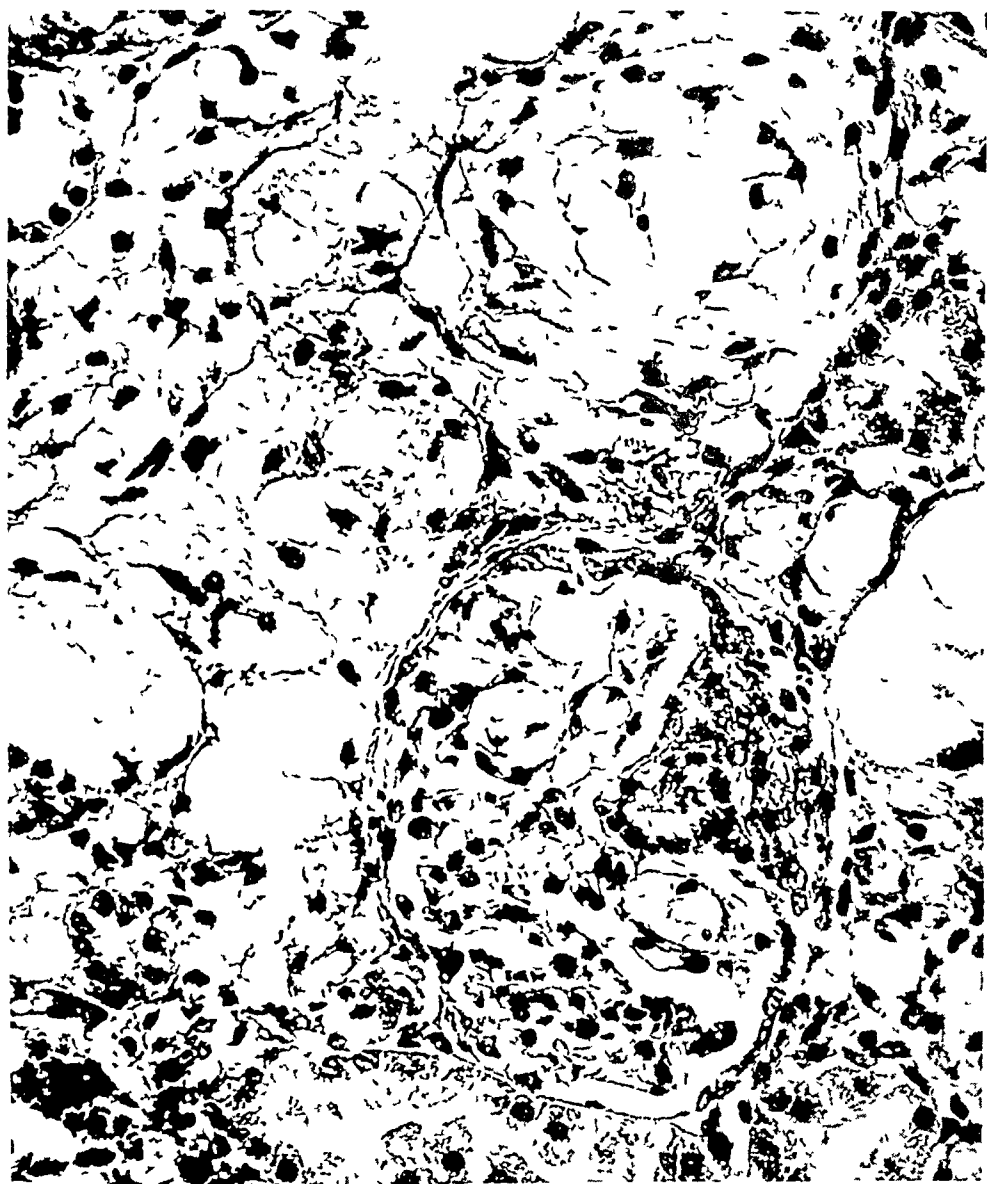


Fig 4—Renal tubules with balloon-like swollen epithelial cells, small cystic cavities and large mononuclear foam cells in the interstitial tissue, a few swollen endothelial cells in the glomerular tuft

**Stomach** There was in most of the rats moderate eosinophilic infiltration of the submucosa of the glandular portion

**Intestine** No abnormalities were observed with the exception of mild eosinophilic infiltration into the submucosa of the duodenum, in a few instances

**Testes** Diffuse, moderate degeneration of the spermatogenic epithelium with desquamation of spermatids and arrest of spermatogenesis was observed in the rat which died spontaneously with severe renal lesions. The testes of the other rats were normal.

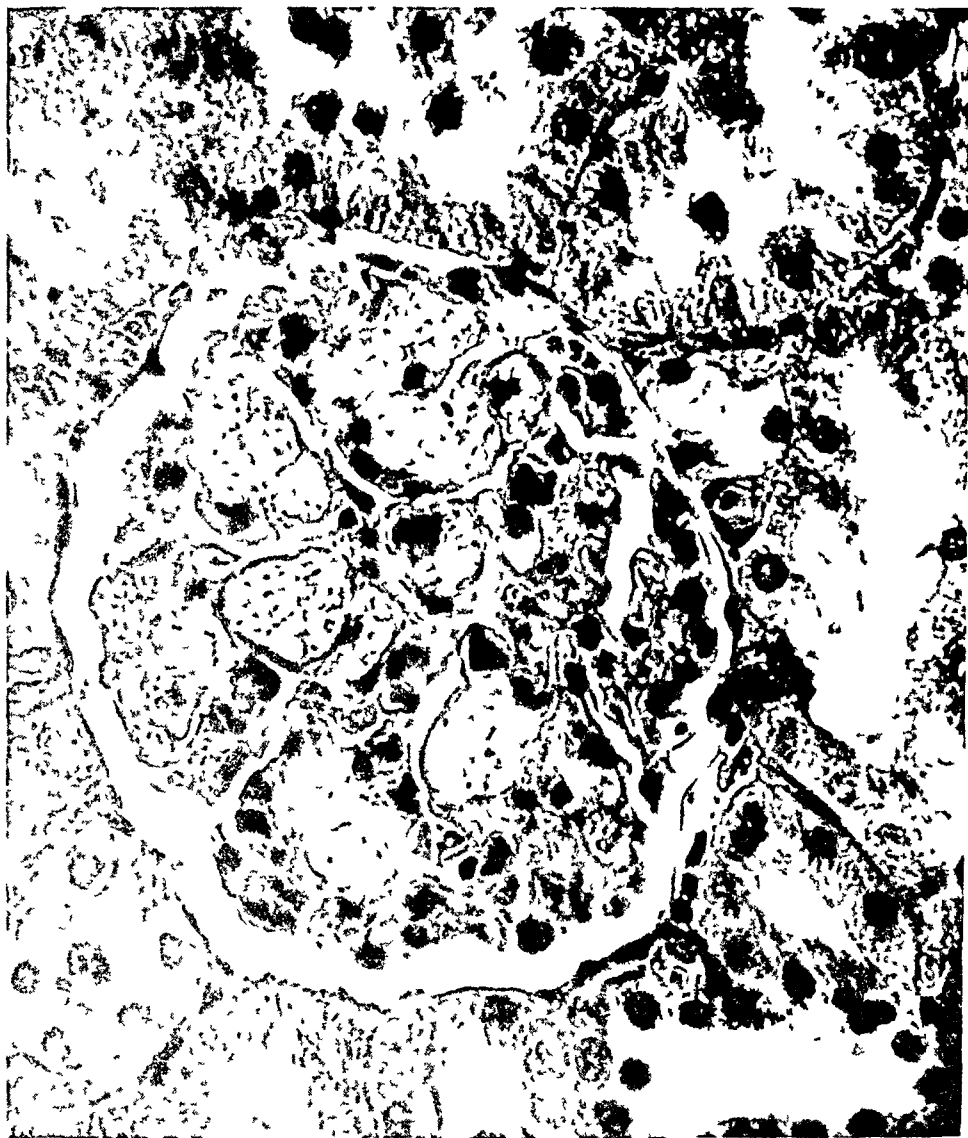


Fig 5—Glomerular tuft with several markedly distended endothelial cells containing polyvinyl alcohol

**Epididymides** The lumens of the ducts of the rat with testicular alterations contained numerous immature spermatogenic cells in addition to some necrotic debris. The epididymides of the other rats were normal.

**Pancreas, Bladder, Prostate and Seminal Vesicles** These were normal in all the rats of this series.

**Bone Marrow** There was dense myeloid marrow containing numerous eosinophilic myelocytes and megakaryocytes in the sternum in the great majority of

rats examined Erythropoiesis seemed to be somewhat impaired in several rats, while small foci of degeneration were scattered throughout the marrow in a rat showing marked lesions in other organs

**Brain** No abnormalities were demonstrable in the brains of 6 rats. In 3 rats large mononuclear foam cells were found in varying number in the stroma of the choroid plexus. Another group of 3 rats showed, in addition to identical choroidal lesions, pathologic changes affecting the blood vessels and the nerve tissue. There were brown-pigmented cells and balloon-like, swollen glia cells in the perivascular spaces of some vessels, which revealed, moreover, proliferation of their endothelial lining. Accumulations of swollen glia cells possessing foamy cytoplasm with fine bluish gray granules were found occasionally near and within a nerve tissue having a spongy appearance or an appearance of containing many small cysts. Small groups of ganglion cells situated near the cornu ammonis, the occipital region and subcortical region were distended enormously and vacuolated. They were surrounded or invaded by glia cells in those instances in which marked degeneration of the affected ganglion cells was apparent from the indistinctness of their cellular outlines and the disintegration of the nuclei and of the tigroid substance (Nissl stains) (fig 6)

In comparing the pathologic alterations described as to extent, distribution and severity it appears that in general these features were more pronounced in the rats which were killed two weeks after the discontinuation of the treatment than in those killed immediately following it.

*Histochemical Color Reaction for Polyvinyl Alcohol*—The demonstration of bluish-tinged homogeneous or granular matter at the site of injection as well as in various remote organs strongly suggested the presence of polyvinyl alcohol in these localities. Attempts were made, therefore, to identify more definitely the chemical character of this substance by some selective histochemical color reaction. The stains methyl violet, polychrome methylene blue and Best's carmine, usually used for the demonstration of amyloid and glycogen, respectively, were tried unsuccessfully. A specific and sensitive color reaction for polyvinyl alcohol, however, was obtained when the observation made by Staudinger, Fiey and Stark<sup>1</sup> in regard to the blue colorization of polyvinyl alcohol after contact with compound solution of iodine was utilized. The method to be described can be applied to frozen sections as well as paraffin and pyroxylin (celloidin) sections with equal success.

The sections were placed in compound solution of iodine from four to sixteen hours (overnight). Then they were dried with blotting paper, to remove the excess iodine solution, and mounted in glycerin. Contact with water and alcohol must be avoided, as the reaction is highly sensitive to these agents, which cause a rapid fading of the blue color. Sections thus treated are light to deep brown, depending on the thickness and the particular type of tissue. The polyvinyl alcohol takes on a deep blue to grayish blue color. The reaction is so sensitive that even minute intracellular deposits of this compound are visualized readily.

Sections of nearly all organs were stained with compound solution of iodine, according to the procedure described and the following observations were made.

**Brain** The content of polyvinyl alcohol in the brain as demonstrated by this reaction varied considerably with different animals. Some brains were apparently free from any polyvinyl alcohol. In a few others the polyvinyl alcohol was restricted in its distribution to the choroid plexus where it occurred in the form of fine blue granules in groups of histiocytes and as large globules in the lumens of the smaller vessels or as an inner coating of their walls. In 3 rats of this series



there was present, on the other hand, extensive involvement of the brain. Blue globules were found in some restricted areas of the meningeal tissue, where they were located in cells and capillary lumens. Blue globules and coating of the wall were observed not infrequently in the intracerebral blood vessels. Glia cells accumulated in distended perivascular spaces occasionally contained fine blue granules. In some areas, balloon-like, swollen ganglion cells contained large blue globules, while swollen and irregularly branching glia cells showed dense, finely granular blue inclusions. In other restricted regions the nerve tissue presented a spongy appearance, with large pale to dark blue-stained homogeneous

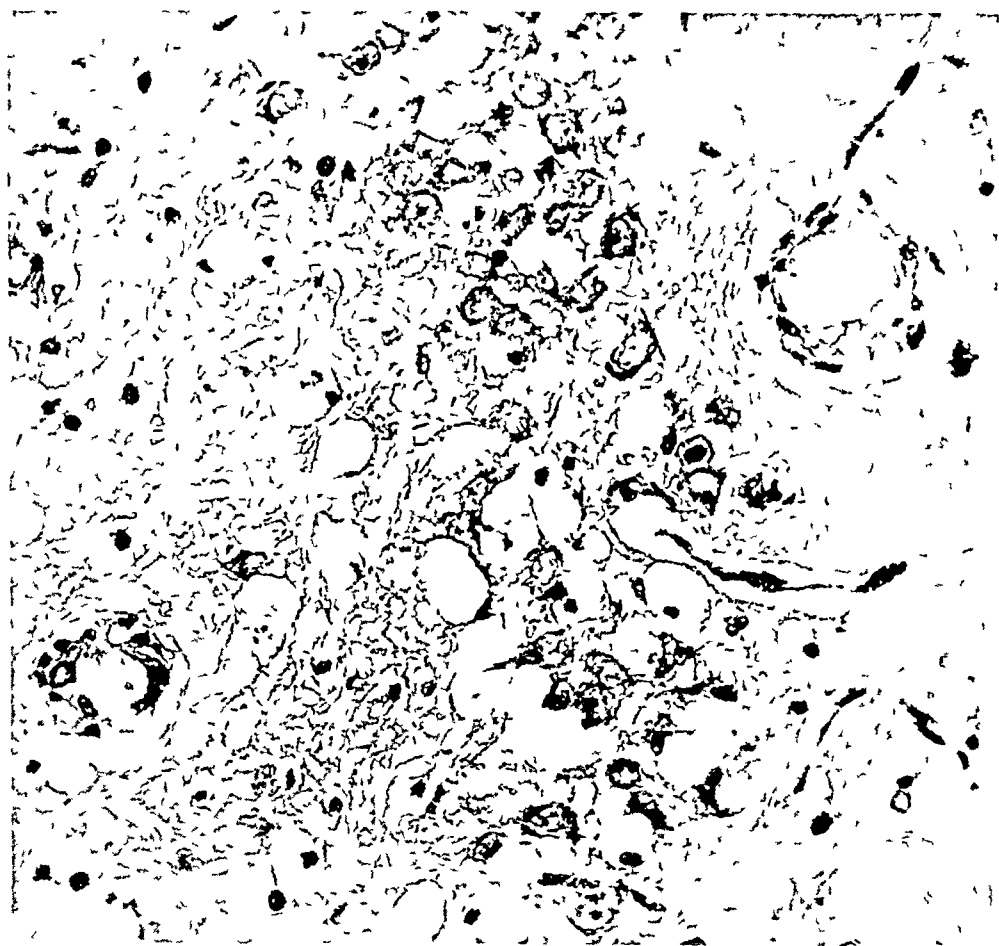


Fig 6—Ganglion cells and glia cells of the region of the cornu ammonis, showing marked swelling, distortion and vacuolation. The vacuoles are filled with polyvinyl alcohol.

masses filling cystic formations surrounded by bundles of nerve fibrils. The extent of these changes appeared to be greater in these specially stained sections than was apparent from the sections stained with hematoxylin-eosin.

**Lungs.** Blue globules were found within capillary lumens and in histiocytic foam cells of the interstitial septums and perivascular spaces. Larger globular blue-stained accumulations were seen in several granulomatous foci, where part of the blue matter was located within foam cells and part formed the center of the histiocytic indurations.

**Heart** In several rats the interstitial tissue of the heart showed foam cell accumulations with fine blue cytoplasmic globulation

**Liver** Smaller and larger blue droplets were present more or less frequently within swollen Kupffer cells. In cases in which these reticuloendothelial inclusions were rather massive, there were observed beadlike bluish streaks running through the brownish-colored hepatic parenchyma. Larger globular accumulations of blue-stained matter occurred either within the foam cells forming the reticuloendothelial nodules or extracellularly within the centers of these foci. Blood vessels showed occasionally an inner coating of the blue substance or contained blue globules within their plasmatic content or had in their lumens accumulations of deep blue-stained round "corpuscles," resembling in size and appearance erythrocytes or large platelets covered with the blue-stained polyvinyl alcohol adsorbed or precipitated on their surfaces. The liver cells proper were always without any blue-stained inclusions.

**Spleen** The amount of blue material demonstrable in the spleen varied greatly with different animals. In the majority there was only a small to moderate number of small blue globules scattered through the pulp, while in 2 rats the entire pulp was studded densely with globular and granular blue matter, located within the masses of foam cells. Blue globules were seen in the vascular and sinusoidal lumens. The follicles were in general free from blue matter with the exception of a few small blue globules within large histiocytes situated in their marginal portion.

**Pancreas** A fine blue granulation was presented in more or less numerous histiocytes of the foam cell type located in the interstitial tissue, while blue globules were seen in the lumens of some blood vessels. Histiocytes of the peripancreatic fat tissue showed not infrequently a fine blue globulation in their cytoplasm.

**Adrenals** The medullary reticuloendothelial cells were loaded with blue globules. These colored inclusions were less dense in the histiocytic cells of the cortex, especially where the subcapsular reticuloendothelial cells revealed these blue aggregations. Small groups of blue granulated foam cells were found in periadrenal fat tissue.

**Kidneys** Gross inspection of the renal sections showed clearly, by the distribution of the blue-colored areas in the brownish yellow matrix, that the polyvinyl alcohol was accumulated principally in the peripheral cortex and in the corticomedullary zone. The region situated between these two areas contained a smaller amount of blue matter, while the pyramids appeared to be entirely free of it. Microscopic examination of the sections revealed in different rats marked differences in the amount and site of blue material deposited in the kidneys. While blue matter was present in the kidney of all rats, in some only a small amount of it was seen, and then it was found almost exclusively within the glomeruli, in the tubular epithelium of the corticomedullary zone and in a few scattered blood vessels. Furthermore, only a smaller or a larger portion of these tissue elements then were thus affected. Kidneys with more massive involvement showed, on the other hand, not only more diffuse and sometimes almost generalized deposition of blue matter in these component parts but often had, in addition, very extensive involvement of the cortical connective tissue.

Blue globules and homogeneous masses were found not only in all those cells and tissues which had shown in the sections stained with hematoxylin and eosin the presence of a faintly blue-colored matter but also in many additional cells and structures, where the last-mentioned sections had not suggested deposition of polyvinyl alcohol. The imbibition of polyvinyl alcohol by the tissue was so

massive in several instances that the original structure appeared to be obliterated. The glomerular tufts contained this substance within the balloon-like endothelial cells as well as free in the capillary lumens. Blue globules were found not only free in the tubular lumens but also within the tubular epithelium.

**Testicles** The extratesticular venous plexus of the rat with well developed degenerative changes of the spermatogenic epithelium contained numerous blue globules as well as an extensive coating of the inner wall with the homogeneous blue substance. Very fine globules were seen in the lumens of many intratesticular small blood vessels. Some of them were coated with this matter for wide stretches, especially in regions where the spermatogenic epithelium in the adjacent tubules showed evidence of marked degeneration. Numerous large oval and polygonal cells in the interstitial tissue were filled with a very fine blue cytoplasmic granulation.

**Bone Marrow** The great majority of the marrows tested showed blue matter in the form of globules or deep blue "corpuscles" arranged either in groups or in irregular chains or clumps. The deposits seemed to lie in sinusoidal lumens and occasionally within large sinusoidal cells. The myeloid cells proper were always without blue inclusions.

**Skin** Examination of the sections by the naked eye revealed a blue to grayish blue zone beneath the epidermis. Dense accumulations of bluish globules within cells and larger solid masses of this substance situated free in the tissue spaces were seen on microscopic study. The balloon-like, swollen histiocytes showed fine cytoplasmic blue granulation. There were also small blue globules in isolated cells and in cell groups surrounding the hair follicles.

Many sections were stained with scarlet red for the presence of fat, especially with reference to the possible occurrence and admixture of fat in the deposits of polyvinyl alcohol in the different organs. The results essentially were negative, with a few exceptions. There was a moderate sprinkling of red-stained granules in the spleen which showed a diffuse infiltration with balloon-like, swollen reticulum cells. The fat granules were located within some of these foam cells and formed occasionally an outer ring around the nuclear crescent of the multinucleated giant cells. The greater portion of the pulp, however, was free of fat. The same animal showed a minor fatty infiltration of the cells in the pericentral hepatic cords and of the tubular epithelium of the kidney.

**Oral Administration**—Only 4 rats were used in this experiment. They were about 2 months old at the start of the experiment. During the first two weeks 2 Gm of polyvinyl alcohol was added to 45 Gm of stock diet. This dose was doubled for a second period of two weeks. Then 2 rats were killed, and the 2 survivors were placed on a diet containing 10 Gm of polyvinyl alcohol in a total of 25 Gm of food (ratio, 1:15). At the end of two weeks these rats were killed.

The rats developed satisfactorily on this diet. They increased their weight within four to six weeks from an original of 78 to 84 Gm to 139 to 172 Gm, respectively.

The necropsy did not reveal any gross lesions of the internal organs.

The histologic examination of sections prepared from the brain, lung, heart, spleen, pancreas, kidney, adrenal, bladder, testis, epididymis, thymus and intestine did not show any abnormalities attributable to the diet given. The livers of the 2 rats which received the largest amounts of polyvinyl alcohol revealed marked hydropic degeneration of the liver cells. There were, however, no proliferations of the Kupffer cells. A more or less marked eosinophilic infiltration was found in

the submucosa of the stomach and in the dense myeloid marrow of the sternum. Sections stained with compound solution of iodine did not show any blue globules or granules indicative of the presence of polyvinyl alcohol.

*Intravenous Injection*—Three male rabbits were used. Each received a daily injection of a 5 per cent solution of polyvinyl alcohol in physiologic solution of sodium chloride (0.85 per cent sodium chloride). The injection was made into the marginal vein of the ear on five days of the week. One rabbit was thus treated ten times with 10 cc of this solution, a second animal received fifteen injections while a third received twenty-five injections, the dose with the last five of them being 20 cc of the polyvinyl alcohol solution. The injections were tolerated in every instance, without any immediate or delayed symptomatic reactions.

The urine, which was examined at varying intervals after the injections, did not contain at any time albumin, sugar or blood. The test for glycuronic acid was positive. The reaction ranged from neutral to alkaline, and the specific gravity varied around 1.010. Examination of the sediment for formed elements revealed none. Urine voided during the first four to six hours following an intravenous injection was collected and saturated with ammonium sulfate. The fluffy white precipitate formed was filtered off and treated with compound solution of iodine. When the deep brown precipitate was placed in water, it changed into reddish blue floccules floating on the surface. The demonstration of this matter in the urine was considered indicative of the presence of polyvinyl alcohol.

A procedure similar to that used for the demonstration of polyvinyl alcohol in the urine was employed in testing for the presence of this substance in the blood. Blood was withdrawn from the heart of 1 rabbit five hours after the last (fifteenth) injection. To 60 cc of the clear amber serum, separated from the clot, 14 per cent sodium sulfate was added. The white soft precipitate settling at the bottom of the vessel was treated with compound solution of iodine. An elastic white network covering the coagulum turned deep blue, thus indicating that a part of the injected polyvinyl alcohol was retained in the blood for as long as five hours after the last injection and was precipitated with the globulins. Polyvinyl alcohol added in the form of a 5 per cent aqueous solution to an equal amount of serum mixed readily without being precipitated and could be recovered qualitatively with the method just described.

The rabbits were killed by intravenous injection of air. Grossly the internal organs were in general normal with the exception of the testes, which were reduced in size, and of the spleen of the rabbit which had received the largest amount of polyvinyl alcohol, which was approximately three times normal size, firm and dirty grayish red, and showed distinctly the grayish white follicles.

In sections stained with hematoxylin-eosin the following microscopic observations were made:

*Brain* There was an occasional small focus of glia cells which were partly balloon-like in character and were located in a loosened fibrillar matrix containing in a few instances vacuolated and degenerated ganglion cells. In 2 rabbits large round foam cells were found in the choroid plexus.

*Lungs* The pulmonary lesions observed in the 3 rabbits were identical in type but varied in degree, depending on the amount of polyvinyl alcohol administered. There were more or less marked and diffuse perivascular and peribronchial eosinophilic infiltrations, usually containing a fair admixture of mononuclear and lymphoid elements. A considerable number of small blood vessels and capillaries were clogged and surrounded by such cellular accumulations. In several more advanced vascular lesions of this type there were small cavities, each with a

central bluish-stained homogeneous mass surrounded by syncytial multinucleated formations and mononuclear foam cell elements mixed with eosinophilic leukocytes. The parts of an endothelial lining preserved in some of them demonstrated that these lesions were actually of vascular origin and had derived from vessels occluded by leukocytic thrombi. A few larger vessels, filled with blue matter, were lined by cushion-like thickenings of endothelial cells, some of which were swollen and exhibited foamy cytoplasm. In a few cases there were multinucleated giant cells intermixed with the endothelial cells forming these thickenings. Occasionally a crescent-shaped portion of the endothelial lining of a blood vessel had been lifted

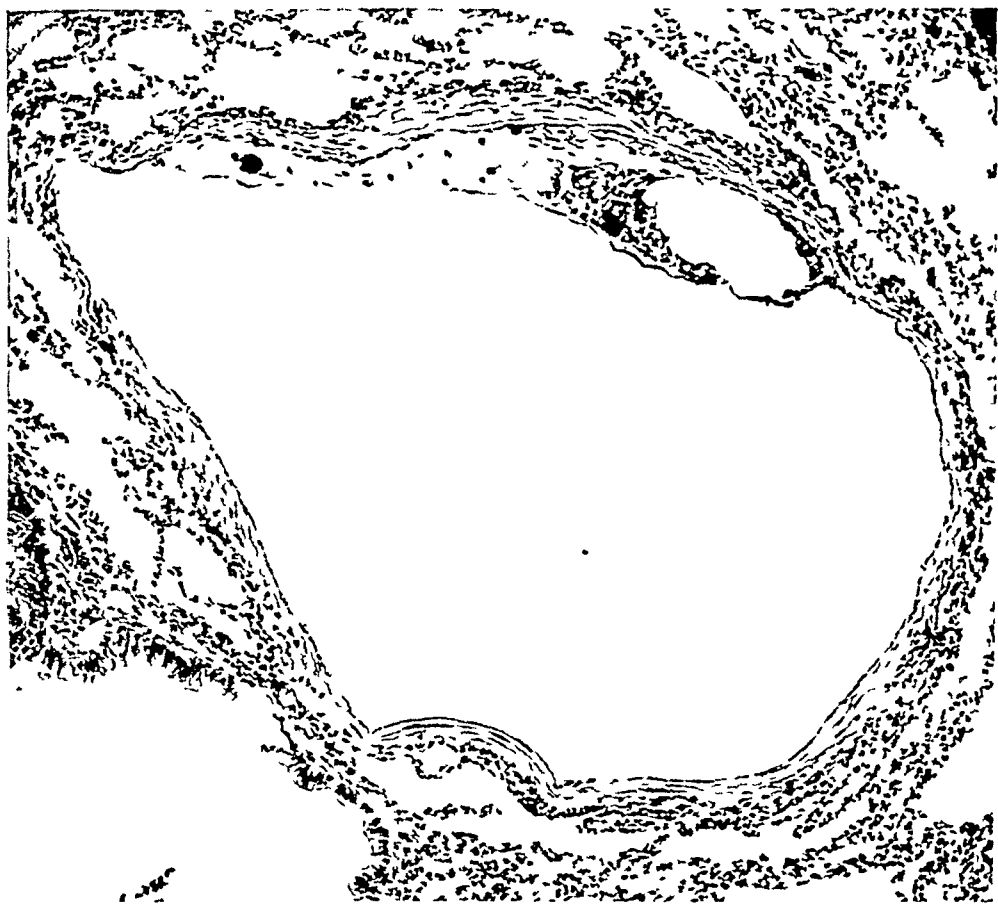


Fig 7—Pulmonary vein containing a crescent-shaped subendothelial imbibition of polyvinyl alcohol

off its base by a highly edematous subendothelial tissue containing a few mononuclear cells, sometimes arranged in a rosette-like cluster (fig 7). Smaller vessels were filled with solid plugs of large pale mononuclear endothelial cells, apparently swollen and proliferated, and were surrounded by dense accumulations of eosinophilic leukocytes.

**Heart** The myocardium was normal. The interstitial tissue contained several small foci of large mononuclear cells located near or within capillaries.

**Aorta** The vessel was normal.

**Thymus** The organ was normal.

**Liver** The liver cells were swollen and pale and filled with a fine granular cytoplasm. The Kupffer cells were apparently normal.

**Spleen** The follicles in general were well preserved. The pulp was hyperemic and contained scattered groups of foam cells in the 2 rabbits which had received the lower doses. The splenic pulp of the third rabbit, which had exhibited the enlarged spleen, presented a massive accumulation of foam cells.

**Adrenals** The medullary reticuloendothelial cells were greatly swollen and partly of foam cell character.

**Kidneys** The kidneys of 1 rabbit were normal, and those of a second animal showed marked degeneration of the tubular epithelium in the corticomedullary zone. The endothelium of the glomerular tufts of the third rabbit presented the typical balloon-like swellings already described, diffuse tubular degeneration and obliterative endothelial proliferations in some interstitial small vessels.

**Testes** There was complete or extensive arrest of spermatogenesis in the testes of all 3 rabbits. The spermatogenic epithelium was moderately to markedly atrophic and thinned. Some tubules contained desquamated spermatids and multinucleated spermatid giant cells.

**Epididymides** The lumens of the ducts were filled with masses of immature round spermatid cells and giant cells, mixed with debris.

**Bone Marrow** The marrow of the sternum consisted of loose myeloid fat marrow revealing impaired erythropoietic activity in all 3 rabbits and reduced myeloid proliferation in 2 rabbits. There were, however, numerous eosinophilic and megakaryocytic cells. The marrow of the rabbit which received the largest dose of polyvinyl alcohol contained several small foci of large interstitial cells with foamy cytoplasm.

Sections stained with compound solution of iodine showed the following changes:

**Brain** Blue granulation was found in perivascular glia cells. It was distributed diffusely in smaller areas of the brain within large foam cells. These were partly glia cells and partly swollen ganglion cells (fig. 8). Blue matter of a spongy appearance, filling the entire lumen, or of a homogeneous type, coating the inner wall of blood vessels, was distributed widely in the brain. Often some of the vessels contained deep blue-stained "corpuscular" matter side by side with brown-stained erythrocytes. There was also blue matter in the veins of the choroid plexus and within large foam cells of its interstitial tissue.

**Lungs** Blue granules were observed in swollen pale cells blocking the lumens of smaller vessels and in subendothelial crescent-shaped spaces. Numerous blood vessels were blocked by homogeneous blue-colored plugs.

**Liver** Only a few Kupffer cells contained small blue globules. The liver cells were free from any blue matter. Somewhat larger amounts of blue matter were seen in vascular lumens.

**Spleen** The pulp contained blue globules in varying number. The deposition of the polyvinyl alcohol in the spleen of the rabbit which had received the largest amount was so abundant that the section had already grossly a bluish color. Reticular cells and sinusoids were clogged with blue masses which involved the entire pulp. In a second rabbit, especially, the subcapsular zone was the site of the blue inclusions and imbibition. Small accumulations of minute blue globules were observed within the walls and in the perivascular tissues of several perforating arteries.

**Adrenals** The reticuloendothelial cells were loaded with blue matter to such an extent that in the medulla blue-colored cells seemed to be the predominating cells. There were dense deposits of blue globules in the histiocytes

of the capsule and of the peripheral cortex as well as in the cortical cells themselves. The blue content of the cortical cells gradually decreased toward the center, following in its distribution the columnar arrangement of the cells in the zona fascicularis. The periadrenal fat tissue showed not only histiocytes containing a fine blue granulation but also fat cells proper with large blue globules.

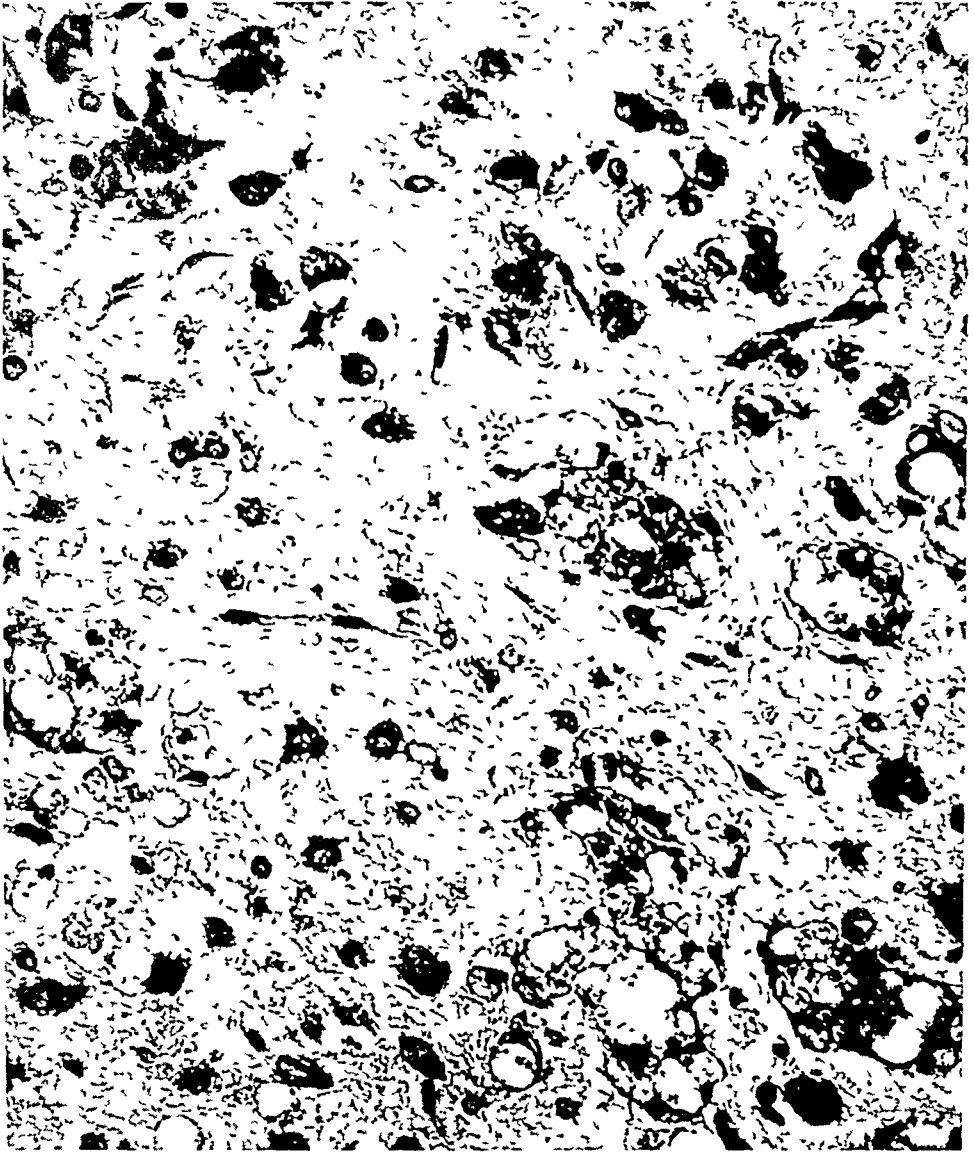


Fig 8—Focus of glia cells and ganglion cells highly infiltrated with polyvinyl alcohol, located in the brain stem

**Testes and Epididymides** Numerous blood vessels contained blue globules or a homogeneous coating or a spongy, honeycomb-like matter. The interstitial cells in the testes were engorged with fine blue granules. The spermatogenic epithelium, however, was always free from blue inclusions. Dustlike blue granules filled the fibroblastic layer of the tunica vaginalis. The histiocytes and fat cells of the peritesticular and periepididymic fat tissue showed numerous blue-colored globular inclusions.

**Bone Marrow** The marrow of the sternum of 1 rabbit only revealed a few blue globules situated within the lumens of sinusoids

Sections stained with scarlet red for the demonstration of fat in general revealed none The presence of fine, dustlike red granules in the interstitial cells of the testes of 1 rabbit formed the only exception to this rule

Inasmuch as the results of a detailed study of the various hematic reactions following intravenous administration of polyvinyl alcohol will be reported in a separate communication, it may suffice to mention here that after several intravenous injections of polyvinyl alcohol there occurred in the rabbits a marked decrease in the coagulability of the blood, a considerable lengthening of the bleeding time and moderate anemia and leukopenia

#### COMMENT

Polyvinyl alcohol subcutaneously administered is retained to an appreciable degree and for an extended period at the site of injection There it causes necrosis and production of a granulation tissue composed of histiocytic foam cells and multinucleated giant cells resembling closely in morphologic character the inflammatory tissue observed after injection of paraffin (Hueper<sup>5</sup>) and unsaturated fatty acids (Hass<sup>6</sup>) The phagocytic cells are active in breaking up and ingesting the masses of foreign material and preparing it thereby for distribution to remote organs by way of the blood stream The lymphatic channels seem to play only a minor role in this process, as the lymph nodes show relatively little evidence that they participate in the mobilization and general distribution of the compound On the other hand, varying amounts of polyvinyl alcohol can be demonstrated within the lumens of blood vessels in various organs and parts of the body The polyvinyl alcohol retained at the site of injection acts as a depot from which over a period of weeks the compound is discharged gradually into the circulation While present in the blood, polyvinyl alcohol is not taken up evidently by the phagocytic leukocytes, but remains extracellular, either finely dispersed in the plasma or forming larger and smaller globules within the plasmatic matter The polyvinyl alcohol shows a marked tendency to coat the inside of blood vessels, especially those of smaller caliber, or to occlude their lumens The latter phenomenon seems to be especially frequent and probably most persistent in the lung, as the walls of the smaller vessels of this organ reveal the most marked and striking evidence of the injurious effect of polyvinyl alcohol lodged in their lumens Following an arrest of leukocytes in the pulmonary capillaries thus damaged, there occurs a swelling of the endothelial cells, with foamy transformation of the cytoplasm by intracellular imbibition of polyvinyl alcohol The subsequent degeneration of the endothelial cells may cause secondary

5 Hueper, W Frankfurt Ztschr f Path **29** 276, 1923

6 Hass, G M Arch Path **26** 956 and 1196, 1938



excessive proliferation of these cells or may result in replacement of the endothelial lining by multinucleated giant cells and histiocytes, while at the same time there is more or less dense perivascular and vascular infiltration by eosinophilic leukocytes and mononuclear cells. The polyvinyl alcohol appears occasionally to seep through the injured endothelial lining and to accumulate in the subendothelial space, forming thereby a myxomatous-like blister. Polyvinyl alcohol penetrating beyond the vascular wall into the interstitial tissue is engulfed by histiocytes, which are transformed thereby into foam cells. The most important and characteristic late lesions produced in the lungs by the retention of polyvinyl alcohol are represented by scattered obliterative pulmonary arteriosclerosis.

The vascular reactions observed in other organs (brain, liver, testes and others) are in general less spectacular with the exception of those seen in the kidney. In this organ the polyvinyl alcohol while passing through the glomerular tuft is retained by the capillary endothelium of this portion of the renal filter and, accumulating in the endothelial cells, causes then more or less marked ballooning and ultimate disintegration. The retention of polyvinyl alcohol in the smaller branches of the renal arteries is followed by an escape of this substance through the damaged walls into the interstitial tissue, where it may cause not only a chronic inflammatory reaction of the foreign body type but also a diffuse and highly destructive imbibition of the alcohol by the tissues. The coating of the walls of the renal vessels with polyvinyl alcohol may also be one of the main reasons for the more or less extensive tubular degeneration present in most of the affected kidneys. The physico-chemical qualities of the substance make it likely that such a film may interfere seriously with the proper exchange of nutritive substances and metabolites between the blood and the cells of the vascular wall as well as of the surrounding tissue.

This mechanism also may account for the degenerative changes found in the testes of 1 rat and the rabbits which showed the vascular coating just mentioned.

The cellular elements chiefly engaged in the removal of the polyvinyl alcohol from the blood and in its subsequent and prolonged storage are the reticuloendothelial cells of the spleen, adrenals and liver and to a minor extent also those of the lymph nodes. The reticular elements of the bone marrow apparently do not participate in this process. The degree of phagocytosis of the polyvinyl alcohol by the reticuloendothelial cells varies considerably with the different animals. In those showing this process there is most markedly developed not only generalized and extensive swelling but considerable proliferation of the reticuloendothelial cells, and with the massive accumulation of foam cells there results a

remarkable change in the morphologic appearance of certain organs (spleen, liver, adrenals)

Fixed tissue cells with phagocytic qualities usually participate in the intracellular storage of the mobilized polyvinyl alcohol. Histiocytic foam cells located in the subcutaneous tissue, in the lungs and heart, in the pancreatic, testicular, epididymic and periadrenal connective tissue and in the choroid plexus contain polyvinyl alcohol in finely granular form. With highly excessive storage the glia cells of the brain also become engaged in the storage of this chemical.

The polyvinyl alcohol does not seem to penetrate into any parenchymatous cells with the exception of the renal tubular epithelium, adrenal cortical cells and occasionally some of the ganglion cells of the brain. While the presence of polyvinyl alcohol in the renal epithelium seems to be mainly a transitory excretory phenomenon, the occurrence of the compound in the cerebral ganglion cells must be considered as the result of active absorption and retention. Fat cells in various parts of the body and fibroblasts of the testicular tunica vaginalis are the only other cells which ingested polyvinyl alcohol.

The organic lesions resulting from repeated intravenous administration of an aqueous solution of polyvinyl alcohol are essentially identical in type and distribution with those produced by subcutaneous introduction of polyvinyl alcohol. It is remarkable, however, that the most severe and extensive lesions occur in the lungs, spleen and testes when the intravenous route is used, while the kidney, liver and spleen are the sites of the most marked changes following subcutaneous administration of this compound.

In analyzing the data presented, it becomes evident that the several physicochemical properties peculiar to polyvinyl alcohol (large molecular size, viscosity of the aqueous solution, precipitability from a colloidal liquid state to a particulate hydrated gel state through changes in the salt concentration of the medium, dispersibility of the aqueous solution in the blood, marked resistance against chemical or enzymatic degradation) in combination are the causes underlying the various organic lesions observed. It seems reasonable to assume that the large molecular size of this compound represents probably the main reason for the difficulties encountered in its elimination through the kidneys. Polyvinyl alcohol, once having entered the organism, is retained, therefore, to an appreciable degree, as it cannot pass readily through the renal endothelial filter. The retained portion of the polyvinyl alcohol is thus stored in reticuloendothelial cells, in various phagocytic cells and in interstitial tissue spaces, as its metabolic destruction or degradation into smaller molecules apparently cannot be accomplished by the body cells. The morphologic changes produced in the spleen, liver and to some extent also the brain possess a certain degree of similarity with those observed

in lipoidoses of the Gaucher and Niemann-Pick types. It is intriguing to speculate on the role which excessive molecular size of the retained and stored lipids may possibly play in the causation of these symptom complexes. One may consider also the possibility that large molecular size combined with the inability of the body to metabolize the formed product is responsible for the deposition of substances such as amyloid.

The absence of polyvinyl alcohol, demonstrable by histochemical methods, in the various organs following oral ingestion of large amounts of this substance supports the conception that the gastrointestinal mucous membrane is not readily permeable to this macromolecular compound. However, it is still uncertain whether this impermeability of the cellular membrane is complete, as it seems to be likely that the degree of polymerization of the polyvinyl alcohol, and therefore the molecular size of the individual aggregates, is not uniform throughout the solution, thus permitting possibly the penetration of the smaller-sized molecules while preventing that of the macromolecular type.

The observations made with the help of the histochemical color reaction indicate that the polyvinyl alcohol remains to an appreciable extent in a colloidal liquid state and is phagocytosed also in this form by the reticuloendothelial cells and various other cellular elements. There exists, however, some evidence suggesting that a part of the polyvinyl alcohol is changed into a finely divided, particulate gel form. This type of intracellular and extracellular deposition is represented by the grayish blue-stained minute granules found free in the tissue at the site of injection as well as within histiocytes in different parts of the body. The chemical character of the highly reflective stringy inclusions in some of the giant cells present in the granulation tissue at the site of injection is unknown. It appears to be possible that, in analogy with the observations made by Hass<sup>6</sup> in connection with subcutaneously deposited unsaturated fatty acids, these formations represent higher polymerization products of polyvinyl alcohol, resulting from the action of the physicochemical conditions and processes of the tissues on this substance. It is remarkable that neither the leukocytes of the circulating blood nor those present in the bone marrow seem to phagocytose the polyvinyl alcohol globules or granules, while these are ingested readily by many other phagocytic cells. Additional study is needed in regard to the nature of the blue-colored "corpuscles" seen in the blood, as these may represent either globular solid precipitations of polyvinyl alcohol or blood cells (platelets or erythrocytes) covered by a surface film of condensed polyvinyl alcohol.

Histochemical investigations with polyvinyl alcohol are facilitated greatly by the fact that this substance can be traced easily in the various tissues and cells by its characteristic and specific color reaction with compound solution of iodine. As the chemical forms with formalde-

hyde a compound insoluble in water and is insoluble in alcohol and xylene, no special precautions are needed in the handling of tissues to be used for the histologic demonstration of polyvinyl alcohol

#### SUMMARY

Polyvinyl alcohol introduced subcutaneously or intravenously into rats and rabbits is retained in the organism to an appreciable amount and for a considerable time

The injected chemical is stored in (*a*) the reticuloendothelial cells of the spleen liver and lymph nodes, (*b*) the endothelial cells of the blood vessels of the brain, lung and kidneys, (*c*) the histiocytes of various organs and occasionally (*d*) the glia cells and ganglion cells of the brain

The inner wall of blood vessels is often covered by a coat of polyvinyl alcohol, which may result, especially in the lung, in damage to the endothelium with development of proliferative and obliterative pulmonary arteriolar lesions

The polyvinyl alcohol present in tissues and cells can be demonstrated readily in sections by its characteristic and specific blue color reaction with compound solution of iodine

The character and extent of the organic lesions produced are related to the physicochemical properties peculiar to polyvinyl alcohol (large molecular size viscosity of the aqueous solution, precipitability from a colloidal liquid state to a particulate solid state by changes of salt concentration, marked resistance to chemical as well as enzymatic metabolic degradation)

# Case Reports

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## PLACENTA ACCRETA

HAROLD H. NORAN, M D, MINNEAPOLIS

"Placenta accreta" refers to abnormal adherence of the whole or of part of the placenta to the uterine wall after labor. The placenta does not become detached spontaneously and resists forceful manual expression. Irving and Hertig<sup>1</sup> found placenta accreta in 1 of every 1956 deliveries, but Kraul, of Vienna, gave its frequency as 1 in every 20,000 births.

At the University of Minnesota the condition was noted in only 1 of about 28,000 postmortem examinations, but only a very small proportion of these examinations (under 5 per cent) were made on women who died shortly after labor. Among about 30,000 routine surgical specimens there was only a single example of placenta accreta. The higher frequency noted by Irving and Hertig may have been due to the inclusion of cases of partial adherence, a condition which is easily overlooked.

This report deals with 2 cases. In the first the condition was observed at postmortem examination, in the second it was disclosed on surgical removal of a uterus.

### REPORT OF CASES

CASE 1—A married woman, 31 years of age, consulted a physician on June 5, 1931, because of "gas on her stomach" and a feeling of abdominal pressure after eating. She had 1 child, 11 years old, and had had no miscarriages. The menses had been regular, with no abnormal pain or bleeding. There was no albumin or sugar in the urine. The physical examination revealed nothing of importance except moderate enlargement of the uterus. Curettage was performed June 16, 1931, but there is no report of a microscopic examination of the curettings. Immediately after the curettage 75 mg of radium was inserted into the uterus and left for twenty-eight hours, giving about 2,100 milligram hours. The menses ceased entirely from that time until the summer of 1936, when a very scanty flow began. In April 1937 pregnancy of six or seven months was diagnosed. On March 21 the blood pressure and urine were normal. On April 18 the systolic blood pressure was 160 mm of mercury, and the urine showed albumin. On April 26 the blood pressure was 170 mm of mercury, and the urine showed a heavy content of albumin.

On May 1 the patient was admitted to a private hospital. A few rales were heard at the bases of the lungs. The uterus was about 1 inch (2.5 cm) above the umbilicus, and there was pitting edema of the ankles. The fetal heart was heard best in the right lower quadrant of the abdomen, the rate was 150 per minute. The clinical diagnosis was preeclampsia. It was decided to induce labor. A Voorhees bag was inserted at 8:30 p. m. May 1 and was expelled at 2:35 a. m. the next morning. This was followed by the immediate delivery of a footling.

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From the Department of Pathology, University of Minnesota.

1 Irving, F. C., and Hertig, A. F. Surg., Gynec. & Obst. **64** 178, 1937.

breech The baby weighed  $2\frac{1}{2}$  pounds (1,134 Gm) and was placed in an incubator The mother was taken to the delivery room for repair of a second degree laceration of the perineum Hemorrhage was only moderate She was put to bed with the placenta retained Two very short convulsions occurred a short time after delivery The blood pressure was about 210 systolic and 120 diastolic At 10 15 a m May 2 she grew worse The blood pressure dropped to 110 systolic and 80 diastolic At this juncture 500 or 600 cc of blood was expressed from the uterus Crede of the uterus failed to express the placenta An attempt to express the placenta manually was unsuccessful At 11 30 a m the blood pressure was 100 systolic and 68 diastolic Large clots of blood were expressed The pulse became weak and irregular A consultant was called With much difficulty he removed part of the placenta and stated that it gave the sensation of being firmly adherent and that no line of cleavage could be found To stop hemorrhage it was necessary to pack the uterus and the vagina An intravenous injection of 2,000 cc of dextrose solution was administered, and later a transfusion of 400 cc of blood was given The blood was obviously compatible, and no transfusion reaction was noted After this she rallied a little At 1 45 p m the respiration became rapid, and she became cyanotic At 2 p m the blood pressure was 120 systolic and 80 diastolic, at 2 10 p m the systolic pressure was 68 A 25 per cent solution of pyridine betacarboxylic acid diethylamide (coramine) and a solution of epinephrine hydrochloride were administered, and she was given inhalations of oxygen with carbon dioxide At 3 10 the blood pressure was 120 systolic and 84 diastolic, at 4 30 the skin became cold and clammy At 5 15 coramine and epinephrine were given, without any response being observed She was perspiring profusely At 9 p m the respirations became gasping Intravenous therapy was impossible because of the collapse of the veins Respirations soon failed, and death occurred at 9 30 p m

Numerous examinations of the urine revealed albumin (4 plus), with a specific gravity varying between 1.015 and 1.035 A few pus cells and casts were constant features The blood revealed a hemoglobin content of 81 per cent and red cells 4,250,000 per cubic millimeter

No noteworthy changes were found at autopsy except in the liver, kidneys and uterus The liver weighed 1,700 Gm, there were multiple small hemorrhages under the capsule On section small hemorrhagic areas were seen, more marked in the right lobe Microscopically, these proved to be areas of hemorrhagic necrosis characteristic of eclampsia

The right kidney weighed 150 Gm, the left, 175 Gm Microscopically, there was found thickening of the capillary basement membranes in the glomeruli, characteristic of eclampsia<sup>2</sup>

The uterus weighed 900 Gm On the posterior surface was a small myoma, measuring about 4 cm in diameter There were also a few very small interstitial myomas The placenta was implanted on the posterior wall of the corpus, its inner surface was rough, and it was so firmly adherent that it could be torn loose only in irregular fragments The right ovary contained a normal corpus luteum

Microscopic study of the placenta and the uterus at the site of attachment revealed the most striking alteration to be a widespread hyaline degeneration of the decidua Only small islands of normal decidual cells could be found For

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<sup>2</sup> Bell, E T Am J Path 8 1, 1932

the most part the decidual cells had assumed a solid homogeneous appearance and cell boundaries had disappeared (fig 1) Some of the chorionic villi among the degenerated decidual cells were also hyaline The spongy layer of the placenta was narrow and inconspicuous Many of the maternal blood sinuses were partially or completely occluded by thrombi Numerous small areas of acute inflammation were found in the placenta There were no significant changes in the myometrium The decidual area was narrowed somewhat in certain areas, but the villi did not actually penetrate the myometrium Scattered small foci of calcification were present in the region of the decidua and chorionic villi



Fig 1—Photomicrograph of normal human placenta Note the spongy layer

CASE 2—A married white woman, 40 years of age, consulted her physician on Jan 29, 1937, because of profuse uterine bleeding of several days' duration. Menses had begun at the age of 14 years and had always been regular but excessive, lasting from five to seven days. Four pregnancies had occurred, all with normal spontaneous deliveries and no postpartum complications. There was no history to suggest a miscarriage. Because of unusually severe menorrhagia, in January 1932 50 mg of radium was inserted into the uterus and left for twenty-four hours. After this treatment there was no menstrual flow until January 1933, after which time the menses were irregular, coming at intervals of three to eight weeks, the menses were not painful. In June 1933 injections of antuitrin (an extract of the anterior lobe of the pituitary) were given one week apart. There was temporary improvement in menstruation.

After Nov 1, 1936, she began to have daily uterine bleedings of small amounts. When seen on Jan 29, 1937, she stated that she had been bleeding profusely for about a week. She stated that to her knowledge she was not pregnant. She complained of pelvic pain, which seemed to be associated with the passage of clots of blood. Physical examination revealed an old appendectomy scar and a mass in the pelvis. The corpus uteri was found to be enlarged to the size it would attain with a three to four months' pregnancy, it was soft and boggy. The cervix showed an old scar and blood escaping from the external os.

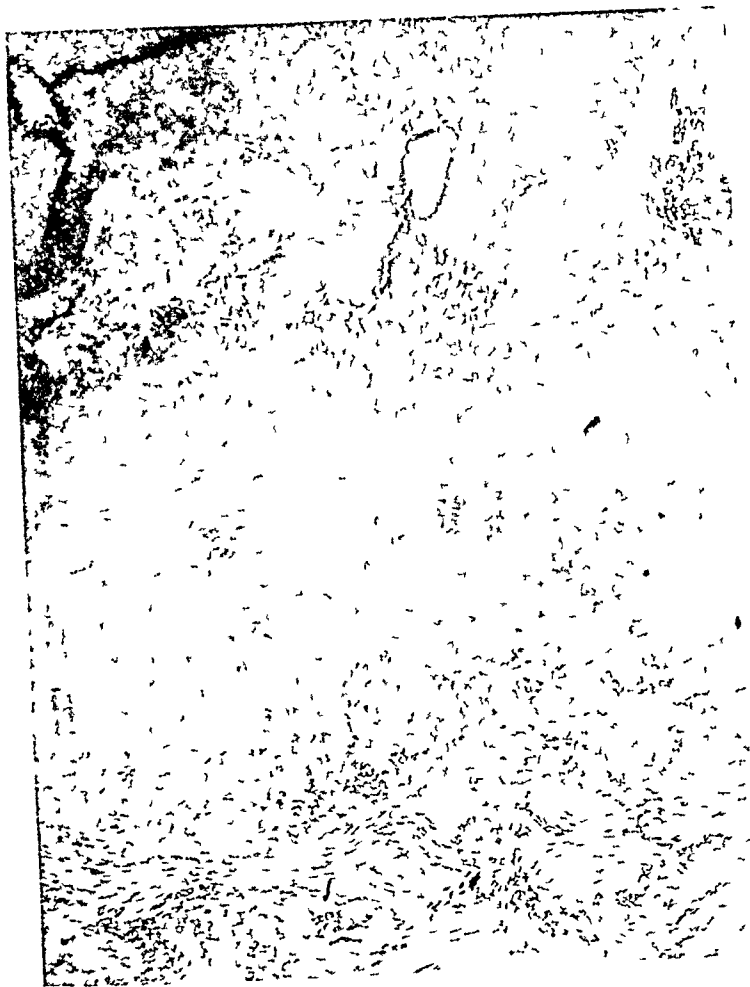


Fig 2 (case 1) —Photomicrograph of a section of the placenta at its site of attachment, showing the hyaline change in the decidua. Foci of calcification are evident.

Numerous urinalyses showed albumin (from a trace to 2 plus) along with numerous red blood cells and a few leukocytes. There is no record as to whether the specimens were obtained by catheter. The hemoglobin content was 51 per cent, the leukocyte count was 24,800, with 91 per cent polymorphonuclear neutrophils. Three indirect transfusions of blood, 500 cc each, were given on January 9, 10 and 17. On January 19 supravaginal hysterectomy was done. The uterus was large and boggy. When it was opened, the cavity was found to be full of clots, and the surgeon saw what appeared to be placental tissue adherent to the wall. The other genital organs appeared normal. The uterus was sent to



the pathologic laboratory at the University of Minnesota. Grossly, it appeared to contain placental tissue, which could be separated from the uterine wall only with much difficulty. It gave the impression that it was firmly attached. When parts of it were forcibly separated, a rough ragged surface was left. In other words, no line of cleavage could be found.

Microscopic study revealed a marked hyaline degenerative change in the decidua, similar to that seen in case 1 (fig 2). The decidual area was still present and was occupied by the hyaline decidual cells. Only a few normal decidual cells could be made out. The spongy layer was completely absent.



Fig 3 (case 2) —Photomicrograph of a section of the placenta at its site of attachment. Note the hyaline decidua and the absence of the spongy layer.

Areas of hemorrhage and inflammation were scattered between the chorionic villi. A narrow zone of acute inflammation was found between the chorion and the decidua and also between the decidua and the uterine muscle. The blood supply to the decidua seemed to be adequate. Areas of subacute inflammation were scattered about in the underlying myometrium.

In the descriptions of placenta accreta in the literature an absence or a decrease in the amount of decidua basalis has been stressed. In the 2 cases presented here a decidual area was found that seemed to be about the same thickness as that observed in sections of normal placenta (fig 3). This also seems to be true of numerous photomicrographs in

various cases, some of which appeared in Irving and Hertig's article on placenta accreta. The most striking change in the decidua in the present 2 cases was hyaline degeneration and not decrease in thickness.

It is well known that the placenta separates along its spongy layer. Contraction of the uterus decreases the size of the placental site. The placenta is relatively inelastic, and it follows that separation must occur if there is a cleavage line that offers only a little resistance. This line is formed by the spongy layer. Thus, if the spongy layer is deficient, spontaneous separation cannot occur, and manual removal will be unsuccessful, because no line of cleavage will be present. This is apparently what occurred in the 2 cases presented. Both showed a definite alteration of the spongy layer.

On considering the underlying etiologic factors involved, the unique feature of the 2 cases is that both patients received intrauterine irradiation prior to conception. Irving and Hertig reviewed the literature of placenta accreta and found no cases in which irradiation of the uterus was received before pregnancy. Hudson<sup>3</sup> mentioned that in many cases pregnancy had occurred after irradiation of the uterus for malignant and nonmalignant conditions, as much as 10,000 mg having been given for the former and 4,500 mg for the latter. No notation is made of placenta accreta. In the present 2 cases it seems that irradiation before pregnancy is the etiologic factor, but, since no similar cases have been recorded in the literature, the relationship may be merely coincidental.

Many investigators believe that previous complications of pregnancy, such as endometritis, retention of the placenta, manual expression of the placenta and cesarean section, are factors which lead to an infectious process which in turn causes the placenta to become adherent. Many cases have been reported in which microscopic study revealed areas of acute or chronic inflammation. The occurrence of eclampsia in case 1 is unusual. Irving and Hertig in reviewing the literature found no record of a case in which placenta accreta was associated with eclampsia.

It is true that the placenta in case 1 overlies a myoma. One theory is that the ovum becomes implanted in atrophic endometrium overlying a myoma and thereby placenta accreta results. In case 1, however, only one corner of the placenta overlies the myoma, so that the involvement was probably too small to cause more than slight atrophy of the endometrium, if any at all.

#### SUMMARY

Two cases of placenta accreta are reported in which intrauterine irradiation had preceded conception. There was no history of abnormality in previous pregnancies in either case. Both placentas showed hyaline degeneration of the decidua and poor development of the spongy layer.

The 2 cases suggest that radium therapy before conception is a possible etiologic factor in placenta accreta, but in none of the many cases of pregnancy following irradiation which have been reported has any mention been made of placenta accreta.

The deficiency of the spongy layer prevents spontaneous or manual separation of the placenta.

# Laboratory Methods and Technical Notes

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## A COMPACT DEHYDRATOR

### A New Device for Dehydrating and Embedding Tissues

GEORGE LUBINSKY, A B, NEW YORK

In preparing tissues for microscopic study, a number of difficulties are met. In laboratories where large numbers of specimens are being prepared every day, a great deal of valuable time is lost in transferring tissues from one solution to another, in corking and uncorking numerous bottles and in cleaning those bottles after they have been soiled with paraffin or pyroxylin (celloidin). Moreover, in pouring solutions out of bottles, bits of tissue frequently are dropped and then must be picked up with forceps, often a damaging process when very delicate tissues are being handled.

Probably the most important difficulty encountered is the poor penetration of tissue when the pieces are a bit over the optimum size and lie on the bottom of a bottle, exposing only one large surface to the fluid. This is especially serious when one is embedding tissue in pyroxylin. In this work very large blocks of tissue are frequent, and thorough dehydration of all parts of the blocks is important.

In an effort to provide ourselves with some method which would overcome as many of these difficulties as possible, my associates and I devised a compact dehydrator, with which we have had excellent results. As will be seen, some of its features are based on devices used in the autotechnicon.

#### DESCRIPTION OF DEHYDRATOR

This dehydrator consists of a series of five round trays, each measuring 3 inches (76 cm) in diameter and  $3/4$  inch (19 cm) in depth, with wire mesh bottoms and perforated metal rims, all nested on a perforated metal stage,  $3\frac{1}{2}$  inches (89 cm) in diameter, which is suspended by three rods from a flat metal cover, 5 inches (127 cm) in diameter. Each tray has removable perforated partitions. The trays are nested so that each forms a cover for the one beneath, and a perforated disk covers the top tray and is held firmly in place by a rod, which is locked by a turn-screw above the supporting disk.

The entire device fits into an ordinary pyrex beaker with a smooth rim, the supporting disk serving as a cover for the beaker. All parts which come in contact with the fluids are made of monel metal. In order to insure thorough washing of the tissues, the dehydrator is placed in a metal beaker with an inflow tube near the bottom and an outlet near the top, thereby giving a forced current through the beaker. Our original model was made to fit into a 1 liter beaker, so that it could be attached to an automatic embedding machine. This necessarily limited the size of the device.

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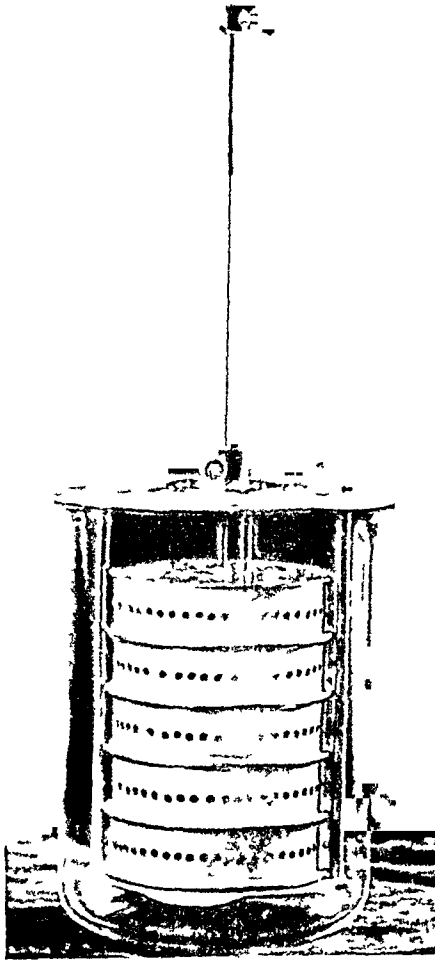


Fig 1—Compact dehydrator showing all of the trays suspended in place within the pyrex beaker



Fig 2—Metal trays, partitions, tray holder, and metal container for washing tissues

For laboratories where large blocks of tissue or large numbers of small blocks are commonly embedded, a larger size which would fit into a 2 liter beaker could be employed to advantage. When very small bits of tissue are to be dehydrated, a lining of extra fine mesh can be inserted in the trays.

#### METHOD OF USING DEHYDRATOR

A short resume of the procedure employed by us in embedding a typical lot of specimens will help to explain our device. The blocks of tissue are placed in the trays and arranged so that they lie flat on the mesh bottom without overlapping each other. The removable partitions make it possible to arrange the tissues from a number of subjects in a single tray. The trays are placed one above another on the platform and are locked into place with the movable lid. The entire device is then placed in the metal beaker for washing. After washing is complete, the dehydrator is lifted out and placed in turn in each of a series of pyrex beakers containing the various dehydrating fluids.

When the paraffin stage is reached, the dehydrator is transferred to the embedding oven, where beakers of melted paraffin are kept. When ready for embedding, the movable lid is raised and the trays are lifted out in turn, and the tissues are embedded. The empty trays are returned to the carrier and are placed in a beaker of waste xylene to remove the paraffin. They are then ready for the next lot of tissue.

Besides the obvious saving in time, we have found that the dehydration of our tissues has been greatly improved, owing to the fact that the penetration takes place through all surfaces of the blocks of tissue, and that a much larger amount of fluid is available (approximately twice as much per block as would be available in an ordinary specimen bottle).

In our laboratory we frequently find it necessary to embed very large blocks of tissue in pyroxylin—for instance, complete cross sections of the brain. In order to facilitate the penetration of these blocks by the dehydrating and embedding fluids and the various mordants employed, we are at the present time working on a variation of the dehydrator which would substitute stationary mesh stages for the removable trays. If found practicable, this device will be reported at a later date.

# General Reviews

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## CULTIVATION OF THE VIRUSES

A CRITICAL REVIEW

MURRAY SANDERS, M D

NEW YORK

The premise is now generally accepted that living cells are necessary for the cultivation of filterable viruses. Tissue cultures and chorio-allantoic membrane preparations have been utilized to this end with varying success. The obviously fundamental importance of this work has attracted many investigators, and claims for the propagation of numerous viruses have been made. It is apparent to any one reviewing the field that there is not infrequently lack of agreement in results and that there is not a sufficiently definite realization of which viruses have or have not been propagated. In my opinion, this is largely due to the fact that there has been no universal acceptance of formulated rigid criteria.

The standards for virus cultivation which I shall describe are not new. They may even appear obvious and their description repetitious. However, they have been determined by the time-tried results of propagating such viruses as vaccinia, yellow fever and fowl plague. If these standards are accepted as the minimal requirements for judging whether there has been virus growth, the number of viruses successfully grown will be seen to be smaller than has hitherto been assumed.

### STANDARDS FOR THE CULTIVATION OF VIRUSES

First, one must make certain that what appears to be propagation of a virus is not survival of the original inoculum. The mere demonstration of the presence of a virus *in vitro* does not constitute demonstration of the growth of that virus. Confusion between survival and propagation may easily be avoided by carrying the virus through a sufficient number of egg passages or culture generations so that the original inoculum is diluted beyond the point of possible infectivity. Absolute rules as to the necessary number of subcultures cannot be given, since the dilution of the inoculum will vary with the amount and the

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In this review superior figures indicate references in the bibliography at the end of the article, superior letters designate footnotes.

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potency of the virus in the beginning and the volume of the preparation into which it was inoculated

Second, an increase in the potency of the virus must occur. Although infectivity after high dilution may be an indication of increased potency, quantitative estimates are desirable. The degree of potency may be determined by cutaneous tests, by inoculation of animals or, with the chorioallantoic preparations, by a count of the lesions produced (Keogh<sup>118</sup>, Burnet and Lush<sup>2</sup>). When these methods are not feasible, it may be possible to demonstrate the degree of potency by neutralization with convalescent serum, as was done in the case of measles (Wenkebach and Kunert<sup>98</sup>).

Third, it is desirable that confirmation of claims for the method and its results should be forthcoming from one or more investigators. As will be seen later, confirmation has been consistent and frequent in regard to those viruses which have undoubtedly been propagated. It is only in cases in which propagation has not been proved that results of investigators seem to be at variance. If this field of investigation is to have permanent significance, methods must be evolved which will permit consistent, predictable results.

Fourth, the formation of inclusion bodies in culture preparations may be considered presumptive evidence of the propagation of a virus. This must necessarily be the least rigid of the criteria, because in some cases inclusions have not been found even *in vivo* and because it is possible to show undoubted cultivation of a virus without the presence of inclusions. However, if inclusion bodies occur, the evidence for propagation becomes so much the stronger.

Fifth, bacterial and viral contamination and spontaneous appearance of viruses should be ruled out by periodic identification of the virus under investigation.

#### METHODS AND MATERIALS

The *in vitro* cultivation of viruses utilizes two types of preparations—the hanging drop and the flask.<sup>1</sup> The hanging drop preparation consists of bits of minced tissue (about 1 mm in the largest diameter) placed in a drop of plasma in the center of a small cover slip. A depression slide, with petrolatum on its rim, is put over the cover slip, forming a small isolated sterile chamber. The whole preparation is then inverted and sealed with paraffin. The simplest hanging drop preparation consists of plasma diluted with a salt solution, and tissue. Further additions of tissue extracts may be made. This type of preparation involves small quantities of tissue and of mediums with correspondingly small inoculums and small production of viruses. However, such a system lends itself to accurate evaluation of specific components and to observation of microscopic changes asso-

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(a) Detailed consideration of tissue culture methods may be found in extensive treatises on this subject by Fischer,<sup>154</sup> Lewis and Lewis<sup>156</sup> and more recently Parker<sup>158</sup>. It is to be understood that all steps in this technic are carried out under strictly sterile conditions.

ciated with the presence of a virus. This is particularly true in studying inclusion bodies. It is also possible to vary the technic by using large Maximow depression slides and to maintain cultures at will.

Flask cultures may consist simply of minced tissue (about 1 mm in the largest diameter) in salt solutions. Tissue extracts and plasma may be added. The flasks most commonly used are the Erlenmeyer, Carrel, "Collar" (see section on variola-vaccinia) and Roux. One advantage of this type of culture is the production of large amounts of virus. The Carrel flask combines some of the advantages of both types of preparations, since it allows microscopic examinations at high magnifications and the production of a large amount of virus.

*Salt Solutions*—The primary function of a salt solution is to provide a diluent containing essential salts in physiologic amounts. As will be seen later, tissues not only survive but also proliferate to some extent in the presence of salt solutions alone. Virus propagation, too, may take place under such simplified conditions.

Tyrodé's solution has been most frequently used in tissue culture work associated with viruses. The formula given below has been taken from Parker.<sup>178</sup>

Sodium chloride	8.00 Gm
Potassium chloride	0.20 Gm
Calcium chloride	0.20 Gm
Magnesium chloride	0.10 Gm
Sodium acid phosphate	0.05 Gm
Sodium bicarbonate	1.00 Gm
Dextrose	1.00 Gm
Water (triple distilled) to make	1,000 cc
Freezing point*	0.62 C

According to Parker, cloudiness and high alkalinity may be avoided by placing about 850 cc of triple distilled water in a 1,000 cc graduate and adding the ingredients in the order listed. The chemicals are thus dissolved completely and distributed throughout the entire volume. When all ingredients have been dissolved, water is added to make 1,000 cc. The  $pH$  should be 7.4 to 7.8. Sterilization is done by passing the solution through a Berkefeld or Seitz filter. Tyrodé's solution cannot be sterilized by heating because of the presence of bicarbonate.

I have found Simms' salt solution very satisfactory. It is made up in two concentrated solutions. Fifty cubic centimeters of each solution is added to 900 cc of double distilled water. The advantage of concentrated solutions is great, since, by making up relatively small quantities of concentrates, a large store of salt solution is insured.

The formula of Simms' salt solution, heretofore unpublished, is given here with the permission of Dr. H. S. Simms, of the department of pathology, College of Physicians and Surgeons, Columbia University.

Solution A	Final Concentration, Gm per Liter	Concentrate, Gm per Liter
Sodium chloride	8.000	160.00
Potassium chloride	0.200	4.00
Calcium chloride ( $CaCl_2 \cdot 2H_2O$ )	0.147	2.94
Magnesium chloride ( $MgCl_2 \cdot 6H_2O$ )	0.203	4.06
Solution B		
Sodium bicarbonate	1.010	20.20
Disodium phosphate ( $NaHPO_4$ )	0.213	4.26
Dextrose	1.000	20.00
Phenol red	0.050	1.00



Solution A is autoclaved. Solution B is filtered through a neutral sintered 5/3 Jena glass filter and kept, stoppered, in the refrigerator. Fifty cubic centimeters of solution A is added to 900 cc of double distilled water. The whole is then autoclaved. Fifty cubic centimeters of solution B is added after autoclaving.

Several months' experience with the Simms' salt solution has shown that tissues are maintained in it to a good degree, that its  $p_H$  remains unusually stable and that it can be used as a diluent for tissue extracts and serum. Concentration of the ingredients in "mother solutions" permits a constant source of supply. The presence of phenol red as an indicator allows observation of the  $p_H$  at all times.

Other salt solutions used by various investigators include Ringer's, Locke's, Drew's and Hartman's.

*Tissue Extracts and Plasma*—Tissue extracts made from whole embryos or from organs (particularly the spleen) have been added to cultures to provide essential growth substances. Carrel and his co-workers contended that embryonic extract has the power of promoting cell growth (Parker<sup>158</sup>) and that only through the addition of such extracts are continuous series of tissue cultures possible. However, in the propagation of viruses, serial cultivation has not been hindered by the omission of tissue extracts. Simms<sup>159</sup> showed that embryonic and spleen extracts may contain substances that inhibit growth.

Plasma provides a permeable coagulum most commonly used for keeping tissues stationary in hanging drop and Carrel flask preparations. Growth-producing factors seem to be present in the plasma, since cells can be grown in plasma diluted with salt solution. Homologous plasma is commonly used but is not essential, particularly if homologous serum is also added. Chicken plasma is satisfactory for tissues from many species.

Serum is frequently added to tissue cultures. In the case of at least one virus, that of yellow fever, serum was found to be important for propagation. Haagen<sup>147c</sup> was able to show that the growth of this virus was greatly reduced when serum was removed from the culture system.

*Tissues*—The all-important cells which provide the living elements for tissue cultures have been largely but not exclusively supplied by embryonic tissues. The problem lies in selecting a host most gracious, and cells most receptive, to the virus.

Is the reaction between virus and host in vitro a specific one? The answer cannot as yet be given categorically. On the one hand, there is evidence that chick embryonic cells provide some common denominator essential or desirable for the propagation of viruses, because many viruses not pathogenic for birds are readily grown in their presence.

On the other hand, there is experimental evidence of definite specificity, for example, the fowlpox virus has been grown in tissue cultures containing chick cells and also on the chorioallantoic membrane, but when mouse tissue replaced chick tissue the virus did not respond (Findlay<sup>3</sup>). Virus III grew in the presence of rabbit tissue but not in the presence of chick cells (Ivanovics and Hyde<sup>144</sup>). The virus of foot and mouth disease, which attacks a large number of natural hosts, has been propagated with ease in cultures containing guinea pig tissue (see section on foot and mouth disease), but egg membrane (Galloway and Elford<sup>20</sup>) and embryonic chick tissue (Matland and Matland<sup>22</sup>) produced no growth.

Recent experimentation with rabies has opened an interesting line of speculation in regard to tissue specificity of viruses. In the past, several investigators reported that rabies did not grow on the chorioallantois, but when Dawson<sup>85</sup> inoculated the virus directly into the brains of the embryos he met with success. What had appeared to be a question of specificity was shown to be a matter of varying the route of inoculation—in other words, a question of technic. Is it not possible that other so-called specific reactions will yield to similar improvements in technic?

Investigators are not in agreement as to the state of cells most favorable to the propagation of viruses. Parker<sup>158</sup> maintained that functional activity is desirable. Plotz,<sup>131b</sup> on the other hand, stated that proliferation was necessary for optimal conditions of virus growth. This agrees with the findings of Carrel<sup>95a, b</sup> for Rous sarcoma. Maitland and Maitland<sup>121c</sup> reported that propagation of a virus (vaccinia) could take place in the presence of nonliving tissue undergoing marked autolysis. However, when Rivers, Haagen and Muckenfuss<sup>132c</sup> repeated that work, cells were found to be alive after five days and to be capable of proliferation after removal from the Maitland medium to cover slip preparations. Zinsser and Schoenbach<sup>160</sup> found that growth of the virus of equine encephalomyelitis occurred as long as cellular respiration increased, but that the rickettsia of typhus propagated as cellular respiration decreased.

Maitland and Maitland<sup>22</sup> noted that the amount of virus growth was independent of the amount of tissue growth. Hallauer<sup>27a</sup> maintained that he was able to propagate the virus of fowl plague for 30 culture generations without tissue growth at 30 C. According to Magill and Francis,<sup>49a</sup> too small or too large amounts of living cells may be detrimental to virus increase. Hecke<sup>21c</sup> found approximately the same rate of virus multiplication when 1 or 10 pieces of tissue were placed in cultures, but when 20 pieces were used, the virus increased at a faster rate.

Concerning the ratio of tissues to fluid medium, it has been suggested by Li and Rivers<sup>121</sup> that 0.1 Gm to 4 to 5 cc is optimal. However, in larger cultures they used 2 to 4 Gm of tissue in 15 cc of Tyrode's solution. According to Plotz,<sup>131b</sup> too much fluid may be harmful, and with little fluid the tissues have a better opportunity for aeration. Parker<sup>158</sup> stated that when tissue cultures are used for the cultivation of viruses the ratio between mediums and tissues should be greater than under normal conditions.

It is apparent that optimal cultural conditions vary with different viruses. Some viruses have the ability to grow under widely varied conditions, while others apparently require more rigidly controlled environments. The virus of vaccinia, for example, appears to grow equally well in a nutritive system such as the chorioallantoic membrane<sup>b</sup> and in a preparation as simple as tissue and Tyrode's solution.

#### AVIAN POX (FOWLPOX)

*Tissue Cultures*—In 1928 Findlay<sup>8a</sup> and Loewenthal<sup>5</sup> succeeded in cultivating the virus of fowlpox for a small number of generations.

Flask cultures containing embryonic chick brain and skin were used by Findlay, who was able to demonstrate by the fourth generation a level of potency 20 times that of the original virus. However, the fourth subculture showed a decrease in titer from that of the second and third subcultures. This work was later repeated by Bierbaum and Gaede,<sup>1</sup> who maintained the virus for 12 culture passages but were unable to transmit the disease to mice.

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(b) The technical aspects of chorioallantoic preparations have not been discussed here. Detailed consideration of this technic can be found in the following studies: Burnet<sup>108</sup>, Burnet<sup>153</sup>, Goodpasture, Woodruff and Buddingh<sup>113a</sup>, Woodruff and Goodpasture<sup>188</sup>.

Although the virus of fowlpox is usually considered as having been successfully cultivated, the evidence indicates that the propagation was only temporary and that the increase in potency was small and unpredictable

*Chorioallantoic Preparations*—The egg membrane has shown more promise for the propagation of fowlpox virus than have tissue culture methods. The virus of fowlpox was the first studied by Woodruff and Goodpasture<sup>138</sup> in 1931. More interested in the pathologic manifestations than in the increase in potency, they made a thorough study of the former and neglected quantitative estimates of the latter. They found that the macroscopic pathologic picture was more easily analyzed when a dilute inoculum was used. In two days small opacities that represented ectodermal proliferation were visible. The lesions were less sharply defined than those of vaccinia and revealed only a minimum degree of inflammation and necrosis. In five days some lesions reached a diameter of 1 cm.

It was the experience of Woodruff and Goodpasture<sup>138</sup> and of Burnet<sup>108</sup> that the virus retained its pathogenicity for fowls after repeated egg passage. Cytoplasmic inclusions (Bollinger bodies) were consistently present in almost every cell in the central portion of the lesion, becoming smaller and less frequent toward the periphery and absent at the edge. Quantitative estimates of increases in potency were made by the pock-counting method<sup>108</sup>.

Specific antibodies, previously shown to exist by Findlay,<sup>3b</sup> were demonstrated by the reaction of immune serums on infected egg membranes (Burnet and Lush<sup>2</sup>). The method whereby the number of lesions on the membrane may be proportionately reduced by contact with immune serum is an important development of the chorioallantoic membrane technic and one which might well be applied to the identification of other viruses.

In the opinion of Burnet<sup>108</sup> vaccine prepared from egg passage fowlpox virus would be efficacious and safe, but it has not been used to date.

#### CORYZA

*Tissue Cultures*—In 1917 Foster<sup>7</sup> inoculated filtrates of nasal secretions into tubes containing rabbit kidney and ascitic fluid covered by a layer of liquid petrolatum. A second culture generation was thought to be pathogenic for man, but this fact was not confirmed.

Since that time the evidence for propagation of the coryza virus has been more definite. In tube preparations containing one half of a minced chick embryo and Tyrode's solution or buffered bouillon (1-2,000 cysteine hydrochloride added) Dochez, Mills and Kneeland<sup>6a,b</sup> were able to maintain the virus for 15 and 17 subcultures and to cause severe

"colds" in human volunteers with material from final subcultures of the series. Powell and Clowes<sup>8</sup> repeated this work, carrying the virus through 31 culture generations, and demonstrated more severe symptoms in volunteers inoculated with later subcultures than in those inoculated with early culture material. Evidence of virus growth was the infectivity of cultures after the dilutions of the original inoculum had reached  $1:10^{27}$  (Powell and Clowes<sup>8</sup>).

Dochez, Mills and Kneeland<sup>9a</sup> provided further confirmation when they obtained severe symptoms by inoculating the fiftieth transfer of a series cultivated under anaerobic conditions. The same generation of a culture grown aerobically caused no symptoms. They reported that transfer of cultures had to be carried out every two to three days to maintain the virus strain.

In view of the definite confirmation, the large number of subcultures and the increases in potency (as measured by dilution of original inoculums), the propagation of a filtrable agent capable of producing symptoms referable to the upper respiratory tract should be acknowledged.

*Chorioallantoic Preparations*—Material from the third generation on chorioallantoic preparations produced moderately severe "colds" in human volunteers (Kneeland, Mills and Dochez<sup>9a</sup>). The original inoculum consisted of 0.2 cc. of a concentrate of a filtrate of material from the nasopharynx of a patient suffering with an acute head cold. A similar amount of ground-up chorioallantoic membrane was used as the passage inoculum. Although no quantitative estimate was made, the great dilution of the original inoculum seemed to indicate propagation of the virus.

Pathologic examination of the infected egg membrane revealed small opaque foci, similar to those described for influenza. In spite of the similarity of lesions, the authors concluded that there was no relationship between the virus which they investigated and that of influenza.

It is apparent that in this case tissue culture methods produced better results than the chorioallantoic technique. Confirmation with more extensive egg transfers is desirable before propagation by this method can be conceded.

#### ECTROMELIA

*Tissue Cultures*—Downie and McGaughey<sup>10a</sup> cultivated the virus of ectromelia for 7 culture generations in preparations of mouse embryonic tissue, mouse serum and Tyrode's solution. The increase in potency of the final culture was estimated at  $6 \times 10^{14}$ . The authors noted degeneration of cells and could demonstrate the virus in both the tissue and the mediums of the cultures. When serum was not added or when embryonic chick tissue replaced the mouse tissue, multiplication of the virus still occurred. However, at no time was the virus pathogenic for young chickens. Inclusion bodies were noted in explanted cells.

The same authors also demonstrated <sup>10b</sup> that immune serum when added to infected cultures did not neutralize or destroy the virus, although increase was hindered.

Although the evidence for propagation appears to be consistent, further confirmation with greater numbers of subcultures is desirable.

*Chorioallantoic Preparations*—The virus of ectromelia was cultivated on the chorioallantois by Burnet and Lush <sup>9</sup> and by Paschen <sup>11</sup>.

Paschen reported 18 successful egg passages. As the number of egg transfers increased, embryonic death occurred more frequently, and pathologic changes in the membrane became more marked. Inclusion bodies could be seen in stretch preparations.

Burnet and Lush stressed the fact that virus growth occurred only if eggs were incubated at 36 to 37 C. They found also a seasonal variation in the effectiveness of eggs. During the spring season the cultivation of the virus was more difficult than in the winter and summer. In the latter seasons passage of the virus could be done at will.

The pathologic changes noted by both groups of authors were essentially similar. Ectodermal proliferation and necrosis were apparent as small opaque foci with occasional satellite forms. If eggs were incubated at 36 to 37 C, death of the embryo was regular and occurred between the third and fourth days. At the usual temperatures of incubation survival of the embryo was the rule.

#### ENCEPHALITIS (JAPANESE B)

*Tissue Cultures*—Successful propagation of the virus of encephalitis (Japanese B) was observed for 16 culture generations in the presence of embryonic chick liver (Haagen <sup>12a</sup>) and for 40 subcultures in the presence of embryonic chick heart (Haagen and Crodel <sup>12b</sup>). The results have been consistent. With a dilution of 1:1,000,000 of the fortieth culture transfer, Haagen and Crodel were able to infect 3 of 3 mice (intracerebral inoculation). However, when rabbit testicle replaced the chick tissue, no growth of virus took place. The authors also tried testicular and splenic tissue from young adult rabbits and mice. The cells were bathed for fifteen minutes in an emulsion of infected mouse brain and then explanted into hanging drops of plasma and embryo extract. In general, adult testicle was better than spleen. No difference in the affinity of cells for the virus was noted among various embryonic tissues.

The large number of subcultures, the consistency of the results and the high dilution at which the cultures showed activity bear weight for the propagation of this virus. Further confirmation is desirable and appears to be only a matter of time.

*Chorioallantoic Preparations*—Haagen<sup>12a</sup> reported 42 egg passages, with increased virulence in the last passage. Haagen and Crodel<sup>12b</sup> infected 3 of 3 mice with a 1:10,000,000 dilution of the seventy-fifth egg generation. They put 3 drops of a 10 per cent emulsion of infected mouse brain into the chorioallantois of eggs incubated for fourteen days and used an emulsion of the membrane for egg passage inoculums. After the twenty-third passage, serum was added to the Ringer's solution as diluent to offset the deteriorating action of this solution. Egg transfers were done every three days. Macroscopically, a fibrinous exudate was apparent on the membrane, but on histologic examination only infiltration with inflammatory cells was noted in the ectoderm and mesoderm. Nuclear and cytoplasmic changes in the ectodermal cells suggested inclusion bodies. The virus was present in the heart, brain, liver, spleen and blood of the embryo, as demonstrated by the infectivity of these tissues for mice, but no pathologic change was noted on microscopic examination of the embryonic tissues.

#### ENCEPHALITIS (ST. LOUIS)

*Tissue Cultures*—The St. Louis virus has been successfully propagated in the presence of embryonic mouse brain by Syverton and Berry,<sup>16</sup> Harrison and Moore<sup>13</sup> and Schultz, Williams and Hetherington<sup>14</sup>. These groups recorded 19, 26 and 16 transplants, respectively. Schultz, Williams and Hetherington reported activity at a dilution of  $10^{-1}$ , but the other two groups agreed on virulence at a dilution of  $10^{-2}$  regardless of which subcultures were tested. With immune serum added to the cultures, mice were protected against infection at dilutions of  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$  (Harrison and Moore<sup>13b</sup>).

When embryonic chick tissue was substituted for embryonic mouse brain, propagation also occurred (Harrison and Moore<sup>13a</sup>). When adult mouse brain was used, only 6 culture generations were successful (Schultz, Williams and Hetherington).

*Chorioallantoic Preparations*—This virus has been passed through 7 and 10 (Harrison and Moore<sup>13a</sup>), 15, 16 and 22 (Schultz, Williams and Hetherington<sup>14</sup>) and 68 egg generations (Smith<sup>15</sup>).

In the recent work of Smith<sup>15</sup> the original inoculum consisted of about 0.1 cc. of an infected mouse brain which was virulent for animals at a dilution of  $10^{-6}$ . There was no evidence of increasing adaptation of the virus to the chorioallantois, and virulence was consistently marked at a dilution of  $10^{-2}$  regardless of the egg generation. Interestingly enough, the brains of mice dying as a result of inoculation with virus from the sixty-seventh egg passage were virulent in exactly the same dilution as the original inoculum, namely  $10^{-6}$ . However, there can be no question of propagation of the virus if notice is taken of the tremen-

dous final dilution of the original inoculum. A possible explanation of the persistence of activity at a dilution of  $10^{-2}$  is that virus growth rapidly attains and keeps a definite level in tissue cultures or in chorio-allantoic preparations.

The pathologic changes noted by Harrison and Moore<sup>13a</sup> were in essential agreement with those observed by other investigators. Cloudy, proliferative lesions with central necrosis and with involvement of all layers occurred in the membranes in four to seven days. No lesions were found in the embryos, although they frequently died, and virus could be recovered from the brain, liver, spleen and membranes. If the chicks were allowed to hatch, some paralysis occurred, and virus was obtained from their tissues. Later, the same authors<sup>13b</sup> demonstrated extensive perivascular cuffing with mononuclears in some of the brains.

#### EQUINE ENCEPHALOMYELITIS

*Tissue Cultures*—The virus of equine encephalomyelitis was successfully propagated by Olitsky, Cox and Syverton<sup>14</sup> in 1934. It was transmitted through 53 culture generations in flasks containing chick embryo and Tyrode's solution. The forty-ninth subculture caused death in mice at a dilution of  $10^5$ . The increase in potency was estimated quantitatively at  $10^{51}$ . The following year Cox<sup>15</sup> using a similar preparation, was able to show that rapid multiplication of the virus occurred in seventy-two hours. He cultured amounts of virus too small to be detected by animal inoculation and after incubation of the cultures found a virus concentration of at least 1/10,000. Lethal animal doses were obtained after further dilution of 1/10,000.

*Chorioallantoic Preparations*—Higbie and Howitt<sup>16</sup> passed ground embryonic brain from egg to egg and succeeded in maintaining a strain of equine encephalomyelitis for 7 and 8 egg passages. Both the chorio-allantoic membrane and the brain of the embryo caused infection in guinea pigs. Grossly, the infected membrane appeared patchy, gray, edematous, thickened and opaque. The virus was recovered from the heart blood of the embryo. The egg was easily used as a means of estimating the neutralizing ability of immune serum.

Covell<sup>17</sup> studied the microscopic changes in chorioallantoic preparations inoculated with this virus and found inclusion bodies in the brains of the chick embryos but not in the membranes.

#### FOOT AND MOUTH DISEASE

*Tissue Cultures*—Definite multiplication in twenty-four hours was noted by Hecke<sup>21</sup> when he cultivated the virus of foot and mouth disease in hanging drops of embryonic guinea pig skin. He maintained the virus for 22 culture generations over a period of one hundred and

thirty-one days. Since tissue necrosis was marked at 37 C and caused destruction of the virus, Hecke incubated the cultures at 30 C and found that the necrotic process was slowed while the virus still propagated. At 37 C the cultures were maintained for only forty-nine days. A parallel series at 30 C was cultivated for eighty-one days. Increased potency was demonstrated by comparing the infective dilutions of 1:1,000 to 1:10,000 of early cultures with the infective dilutions of 1:10,000,000 of later cultures. Virus propagation also occurred when adult guinea pig testis was used, but it was slower and lasted only during 8 culture passages. Fetal lung allowed virus increase through 15 culture generations over a period of seventy-six days. Hecke<sup>21b</sup> later repeated this work with similar results.

Maitland and Maitland<sup>22</sup> failed to propagate the virus in cultures of chick embryo in Tyrode's solution and chicken plasma. However, when the contents of a vesicle from an infected guinea pig were inoculated into cultures of the pads, lips and tongue of a guinea pig, cultivation of the virus was observed through 17 subcultures. The last subcultures, representing a dilution of the original inoculum of about  $4.8 \times 10$ , were infectious<sup>29</sup>. They were stored in the refrigerator for three months, and further subcultures were then made, which showed an increase in virus after incubation in 50 cc of medium in Roux bottles. The highest titers 1:10,000 were observed after three or four days of incubation. Four guinea pigs which had recovered from infections induced by cultures were immune to normally infective doses from guinea pigs vesicles. It was also possible to cultivate the virus in embryonic guinea pig kidney and, to a lesser degree, in adult guinea pig kidney.

Hecke<sup>21c</sup> found that the virus of foot and mouth disease was much more closely combined with the cells than with the fluid components of these preparations. This finding was confirmed by Strieglei,<sup>23</sup> who was able to cultivate the virus through 62 passages over a period of three hundred and sixteen days with an estimated increase in potency of  $10^{11.3}$ . He noted that the virus survived in Drew's solution for three weeks, if glycerin was added, this period was extended eight weeks.

*Chorioallantoic Preparations*—Galloway and Elford<sup>20</sup> showed that after inoculation into the chorioallantoic membrane the virus did not survive twenty-four hours and that the embryo was unaffected. They made use of this fact in establishing a quick effective method for distinguishing the virus of foot and mouth disease from vesicular stomatitis.

#### FOWL LEUKOSIS

*Tissue Cultures*—Furth and Stubbs<sup>24b</sup> in 1934 were the first to report passage of the virus of fowl leukosis in tissue cultures. Leukemic cells were cultured in Carrel flask preparations which contained plasma,



embryonic extract, chicken serum and Tyrode's solution After 10 passages a supposedly pure strain of chicken sarcoma was inoculated into chickens and caused erythroleukosis, the authors felt that two strains of leukosis had been simultaneously cultivated It was noted that the ability to produce erythroleukosis decreased with the disappearance of primitive blood cells

Temporary maintenance of chicken leukemic cells was reported by Verne, Oberling and Guein<sup>25</sup> Hanging drop and Carrel flask preparations were used, but loss of virulence in nineteen and fifteen days, respectively, was detected as the cultures became fibroblastic in character

Furth and Breedis<sup>24a</sup> experimented with various leukemic strains in preparations of chicken plasma, Tyrode's solution and embryonic extract Transfers were made once a week A strain of myeloblastic leukemia after 7 culture transfers infected about 50 per cent of the chickens inoculated and multiplication of the virus occurred only in the presence of round cells (myeloblast-like) The virus was maintained at least sixty-two days

The same authors were able to produce osteochondrosarcoma and lymphomatosis in 1 of 3 chickens They used a strain which had been maintained in cultures for ninety-one days Partial success occurred with leukotic spleen tissue passed through 5 culture generations They were also able to maintain a strain of sarcoma-leukosis for sixty-seven and one hundred and fifty-eight days

As a result of their experiments Furth and Breedis stated that "oncogenic viruses multiply *in vitro* only in presence of cells on which they confer neoplastic properties" If such a view is correct, the propagation of this group of viruses resolves itself into a problem of the cultivation of neoplastic cells From the evidence to date, propagation has been only temporary

#### FOWL PLAGUE

*Tissue Cultures*—In 1931 Hallauer<sup>27a</sup> reported cultivation of the virus of fowl plague for 9 subcultures over a period of eighty-eight days Because he felt that virus growth had occurred at 8 C, a temperature which obviated viability of cells, he later<sup>27b</sup> attempted to establish growth in a cell-free medium but was unsuccessful Increased virulence was demonstrated when embryonic chick brain was used in Carrel flask preparations The highest concentration of virus occurred during the fourth day of cultivation and was followed by a rapid loss of potency However, when the Maitland type of culture was used, virus growth was more gradual and fall in titer slower

Using Erlenmeyer flask preparations of chick embryonic tissue in Drew's solution, Plotz<sup>28a</sup> was successful in passing the virus through 15 culture generations with dilution of the original inoculum to 248-9

Plotz and Ephrussi<sup>25b</sup> demonstrated the ability of normal tissue to proliferate if transferred after seven days from the fluid medium to hanging drop preparations. However, if tissue from infected flasks was taken, proliferation rarely occurred because of cellular damage wrought by the virus. The same authors<sup>25c</sup> also showed that the virus of fowl plague was able to propagate in the presence of cells which were viable but not in a state of proliferation. Under such circumstances tenth passage virus in a dilution of 1:1,000,000 was able to infect and kill a chicken.

By using a strain of virus cultivated on embryonic chick liver cells, Hallauer<sup>27</sup> was able to immunize chickens in two weeks against massive doses of virus. The liver tissue appeared to have the faculty of lowering virulence without reducing antigenicity. The inactivation of the virus in these cultures depended on the amount of inoculum, the degree of cellular proliferation and the duration of cultivation. In vitro experiments with immune serum<sup>27c</sup> yielded results similar to those reported by Rivers, Haagen and Muckenfuss with vaccinia. When immune serum was added to the explanted tissue before the addition of virus, neutralization of the virus occurred, when it was added after the cells had been in contact with the virus, little or no neutralizing effect was observed.

Plotz<sup>25b, c, d</sup> reported that the virus of fowl plague in tissue cultures increased 50 times in twenty-four hours and 100 times in forty-eight hours. After 28 culture transfers a dilution of 1:1,000,000 was fatal to chickens. He found also that too much or too little tissue in the cultures inhibited the formation of virus. In another series of experiments<sup>25e</sup> he noted that embryonic canary was as favorable a medium for this virus as embryonic chick.

By 1934 Plotz<sup>25f</sup> had cultivated a strain of virus for two and a half years, making subcultures every three days. And in 1937 he reported propagation of the strain for five years, through 250 culture generations<sup>25h</sup>.

As is apparent from the evidence, the virus of fowl plague can be grown at will, under known conditions, with predictable definite results.

*Chorioallantoic Preparations*—Burnet and Ferry<sup>26</sup> reported passage of this virus through chorioallantoic preparations. The primary purpose of their investigation was to differentiate the virus of fowl plague from that of Newcastle disease. No reference was made to the number of egg passages, but apparently no difficulty was encountered in transmitting the disease from egg to egg. There was no difference in action between the Brescia and African strains of this virus.

Within fourteen to eighteen hours after inoculations of the chorioallantois the embryo died, death could be delayed slightly by diluting the inoculum. Aside from an increase in moisture, slight edema and an

occasional opaque area, no characteristic changes in the membrane were noted. The embryo showed generalized congestion, with some diffuse hemorrhage into the skin. The principal pathologic differences between fowl plague and Newcastle disease (in egg preparations) appear to be the characteristic distribution and the circumscribed appearance of the hemorrhages produced in the embryo by the latter disease.

#### HERPES SIMPLEX

*Tissue Cultures*—Propagation of the virus of herpes simplex through 10 culture generations was reported by Parker and Nye<sup>37</sup> in 1925. These authors used an emulsion of infected rabbit brain or testis as the original inoculum in a hanging drop which contained normal rabbit testicular tissue in plasma from an infected rabbit. The virus was not demonstrable after the tenth subculture, and no quantitative estimate of increased potency was made.

Confirmation of the first success with this virus has been ample, and in most cases similar preparations containing rabbit testicles have been used (Gildemeister, Haagen and Scheele<sup>33</sup>, Andrewes<sup>29</sup>, Saddington<sup>39</sup>, Haagen<sup>35a</sup>). Virus propagation, however, has also occurred in the presence of chick embryonic tissue (Haagen<sup>35b</sup>) and rabbit cornea (Mitamura, Kitaoka, Watanabe and Ohkubo<sup>36</sup>).

Although herpes simplex appears to offer no great difficulties for *in vitro* cultivation, only one investigator (Haagen<sup>35a</sup>) was able to show the virus present after 60 culture generations over a period of seven months. Other investigators reported 10 (Haagen<sup>35b</sup>), 22 (Gildemeister, Haagen and Scheele<sup>33</sup>), 23 (Andrewes<sup>29</sup>), 25 (Saddington<sup>39</sup>) and 15 (Mitamura, Kitaoka, Watanabe and Ohkubo<sup>36</sup>) successful subcultures. The question which presents itself is why propagation cannot be carried on indefinitely. The possibility suggested by the author's experience is that the dilution in culture to culture passage may be too great. If large transfers were made in late subcultures, more extensive propagation might occur.

The maintenance of virulence and the presence of inclusion bodies in herpes cultures appear to run hand in hand. Gildemeister, Haagen and Scheele<sup>33</sup> found no inclusion bodies in tissue cultures and noted attenuation of the virus and loss of neurotropism. Andrewes,<sup>29</sup> on the other hand, demonstrated inclusion bodies in cultures similar to those in testis and other organs of the living infected rabbit. At the same time he found that his strain did not lose its neurotropic properties but reached a level of potency of at least  $1.4 \times 10^{-28}$ . The presence of inclusion bodies *in vitro* was confirmed by Rivers, Haagen and Muckenfuss<sup>38</sup>. Andrewes also reported that the virus propagated and formed inclusion bodies in cultures of immune testis and normal serum. However, if immune serum was put into the cultures before virus

was added or together with it, no inclusion bodies were formed. In the absence of immune serum the virus infected normal tissues within one-half hour at 17.5 C or at 37 C. It propagated and formed inclusions in spite of a subsequent addition of immune serum. The virus was found to infect immune tissues within forty-five minutes at 37 C.

*Chorioallantoic Preparations*—The virus was first inoculated into chorioallantoic preparations by Dawson.<sup>11</sup> Small nodules were seen on the membrane within thirty-six hours, but only one reinoculation was made. Proliferation and necrosis characterized the pathologic picture, and intranuclear inclusions were present. Other nonspecific cellular changes occurred. When Burnet<sup>10</sup> attempted to repeat this work with two well known herpes strains, the membrane showed weak lesions without visible inclusions.

Saddington<sup>39</sup> reported 24 egg passages with maintenance of virulence in the final generation. Transfers were made every four days, and virulence of the membrane was tested by inoculation into the corneas of rabbits and the brains of mice. This work was later repeated by Burnet, Lush and Jackson,<sup>30</sup> who passed herpes strains through 50 generations of eggs and noted increased pathogenicity for the embryos with extended passage but, at the same time, decreased virulence for animals. Specific intranuclear changes occurred, but these changes were different from the typical intranuclear inclusions.

#### HERPES ZOSTER

*Tissue Cultures*—Glaubersohn and Barg<sup>11</sup> in 1934 inoculated the contents of herpetic vesicles into hanging drops made up of chick embryo heart tissue, rabbit plasma and Tyrode's solution. After incubation for four days at 37 C and two days at room temperature, the culture material was inoculated into the arm of an infant. The results were one positive reaction, i. e., vesicle formation, and one negative reaction. No subcultures and no estimate of increases in potency were made. As Glaubersohn and Barg suggested, the presence of virus in the tissue cultures may have been due to survival of the original inoculum.

*Chorioallantoic Preparations*—In 1936 de Castro Teixeira<sup>40</sup> inoculated the contents of herpetic vesicles into chorioallantoic membranes of twelve to thirteen day eggs. He reported 3 egg passages in which yellowish plaques with depressed centers and irregular contours occurred at the point of inoculation. At more distant points pinhead-sized lesions were disseminated. The potency was not titrated nor was the virus identified. Confirmation by use of this technic has not been forthcoming. On the basis of available evidence, the zoster virus appears not to have been propagated.

## HOG CHOLERA

*Tissue Cultures*—The virus of hog cholera was propagated temporarily in tissue culture by Hecke<sup>42</sup> in 1932. Various hog tissues were explanted in both hanging drop and flask preparations. Because of the predilection of the virus for tissues rich in endothelium, cultures were made up of choroid plexus, lymph nodes, bone marrow, spleen and kidney. Hanging drops of choroid plexus in plasma and spleen extract were infectious for 15 culture passages. Flask cultures of bone marrow in plasma and Drew's solutions maintained virulence through 10 transfers, while lymph node tissue in similar preparations showed the presence of virus after 20 passages. Lymph node cultures were grown over a period of one hundred and five days, and increased potency was demonstrated by infectivity after a dilution of the original inoculum of  $10^{30}$ . There was a dilution of the original virus of  $10^{-9}$  in 14 generations of spleen (flask) cultures. In controls, which contained virus without cells, virulence was lost after eighteen days. Transfers made of the controls showed loss of virus in the second transfer. All cultures and controls were incubated at 37 C.

## INCLUSION BLENNORRHEA

Working independently, Bialek and Thygeson<sup>43</sup> attempted to cultivate the virus of inclusion blennorrhoea in Carrel flask preparations of human conjunctival tissue, Tyrode's solution and human serum. The results were uniformly negative. Chorioallantoic preparations were tried by Thygeson, with no results.

## INFECTIOUS BRONCHITIS OF CHICKS

*Chorioallantoic Preparations*—Beaudette and Hudson<sup>44</sup> passed through chorioallantoic preparations a virus that produced fatal bronchitis in chickens which had been vaccinated against infectious laryngotracheitis. Berkefeld filtrates were used as the inoculums. Fourteen egg passages were reported, with an apparent increase in virulence. From the seventh generation on, the mortality of the embryos increased and the period of incubation decreased. The chorioallantoic membranes did not show lesions comparable to those of infectious laryngotracheitis or of the poxes. No histologic studies were done.

## INFECTIOUS LARYNGOTRACHEITIS OF FOWLS

*Chorioallantoic Preparations*—Burnet<sup>47a</sup> passed the virus of infectious laryngotracheitis of fowls through 7 egg membranes but found a decrease in virulence. When he repeated the experiments with twelve day instead of ten day eggs, the results were more indicative of virus propagation<sup>47b</sup>. This time he was apparently able to pass the virus through eggs at will.

Brandly<sup>46</sup> repeated the work of Burnet and found the chorioallantois to be a good source of vaccine. When immune serum and virus were mixed together, no lesions appeared on the membrane. The potency of the cultivated virus varied directly with the number of visible lesions. These findings were confirmed by Beaudette in 1937.<sup>45</sup>

Investigators are essentially agreed as to the pathologic manifestations on the egg membrane. Within twenty-four to thirty-six hours there were seen on the chorioallantois minute grayish foci, increasing in number to the sixth day. Some opacities became 3 to 5 times as thick as the normal membrane. They were surrounded by characteristic "double-zoned" plaques (Burnet) and occasionally showed central necrotic depressions. The degree of edema varied. The pathologic process was characterized at first by proliferation and infiltration and later by necrosis and degeneration. Death of the embryo occurred in two to twelve days. As the number of egg passages increased, the time of survival of the embryo decreased. Generalized edema and anemia of the embryo were common.

One of the principal features of the disease caused by this virus was the formation of inclusion bodies, which occurred frequently in the ectodermal layer. The following description is taken largely from Burnet's monograph on the egg membrane.<sup>108</sup> Inclusions were present for from twenty-four hours to as long a time as the virus was maintained on the chorioallantois. They developed first as small intranuclear granules. At the same time the nucleolus was enlarged. The cells containing intranuclear inclusions degenerated. Usually, the nuclei became enlarged, the nuclear membrane indistinct, and the cytoplasm granular and pale staining. Liquefaction and ballooning of cells occurred. In younger eggs the necrosis was occasionally more marked, and areas of ectoderm, in which the majority of nuclei contained inclusions, appeared to die and to be separated from the inflamed underlying mesoderm. Inclusion bodies were not seen in the mesoderm but sometimes occurred in the endoderm if the inoculation was made beneath the chorioallantois.

#### INFLUENZA

*Tissue Cultures*—The virus of influenza was cultivated *in vitro* by Francis and Magill<sup>49</sup> by inoculating lung tissue from infected mice into flasks of Tyrode's solution and chick embryo. The tenth culture generation produced infection in mice. Virulence was maintained for 20 successive subcultures. When serum from ferrets which had recovered from this disease was injected into the mice, the culture material failed to cause pulmonary lesions. The same authors<sup>49a</sup> repeated this work with the viruses of human and swine influenza and found the greatest concentration of virus occurring in thirty-six to forty-eight hours, with a decrease in potency in the next twenty-four hours. A swine influenza

strain was cultivated for 70 generations and the Philadelphia human strain for 45. Ferrets and mice succumbed to culture dilutions of 1:1,000 and 1:10,000, and in recovered animals a high concentration of antibodies was noted. Mice were successfully immunized by subcutaneous and intraperitoneal inoculation with the culture material. Antibodies were apparent in persons who had been inoculated with cultured virus.

Smith<sup>50</sup> started with a strain of virus which had been cultivated in chorioallantoic preparations and passed it through 6 generations of tissue culture preparations similar to those used by the aforementioned investigators. He found that the virus was much more closely associated with the tissue than with the fluid component of the cultures.

*Chorioallantoic Preparations*—Successful propagation of the virus of influenza on egg membranes for 10 and 14 egg passages was noted by Smith<sup>50</sup> and Burnet,<sup>48a</sup> respectively.

According to Burnet, 3 of 4 eggs revealed definite characteristic lesions, which on macroscopic examination appeared as small opaque edematous foci, about 0.5 mm in diameter. The central opaque spots were frequently surrounded by lighter halos. Microscopically, slight focal thickening of the ectoderm, with occasional necrosis of cells, was seen. The superficial cells were somewhat necrotic, while the deeper cells underwent proliferation. The height of the infectious process occurred in forty-eight hours. It was followed by reparative activity, which led to cornification of cells near the areas of necrosis. In the opinion of Burnet, the changes preceding necrosis were not specific. Eosinophil migration to the membrane was also noted.

Burnet<sup>108</sup> and Burnet and Lush<sup>48b</sup> observed that the pathogenicity of the influenza virus for egg preparations became more marked after many passages. Burnet found that after the fifty-second passage the embryos were killed between the fourth and fifth days of incubation and that after the sixty-third passage death occurred on the third day. Dead embryos at first showed only congestion of the abdominal viscera, but later hemorrhagic encephalitis was present. By the seventy-sixth passage multiple hemorrhages were noted in the muscles, skin and brain.

Burnet and Lush<sup>48b</sup> compared the W/S and Melbourne strains, which had become adapted to egg preparations by repeated passages. These strains were equally virulent for embryos but differed in virulence for animals. Unlike the Melbourne strain, the W/S strain was pathogenic for ferrets and mice. Good immunity occurred regularly in mice inoculated with the Melbourne strain.

The ability of four groups of investigators to pass the influenza virus consistently through eggs, coupled with the clearcut reactions between cultivated virus and immune serums, testifies to definite propagation on

the chorioallantoic membrane. Although the evidence for cultivation *in vitro* is less striking, it indicates at least temporary growth.

#### KIKUTH'S CANARY VIRUS

*Tissue Cultures*—In 1933 Heizberg<sup>52</sup> propagated Kikuth's canary virus in a tissue culture preparation of embryonic chick in Tyrode's solution. The original inoculum was a single drop of blood from the heart of an infected canary. After ten days the culture material produced an infection in canaries. Virulence was apparent after 10 culture generations, and the presence of inclusion bodies in the cultures was also noted.

*Chorioallantoic Preparations*—In the same year Burnet<sup>51a</sup> was able to cultivate the canary virus on the chorioallantois with little difficulty. Egg passages were made every five days, and 8 transfers were done. The lesions of the membranes, as might be expected, were similar to those seen in the bird poxes. Ectodermal proliferation in the form of opaque patches in the membranes was noted along with some necrosis and inflammation. Large cytoplasmic inclusion bodies were frequently seen. Burnet and Lush<sup>51b</sup> confirmed the fact that this virus could be grown on the chorioallantois and compared the canary virus and the virus of fowlpox serologically.

#### LOUPING ILL

*Tissue Cultures*—Rivers and Ward<sup>54</sup> were successful in passing the virus of louping ill through 11 subcultures in preparations of chick embryo, monkey serum and Tyrode's solution. The original inoculum was a 10 per cent emulsion of infected mouse brain. The fourth and eighth subcultures in dilutions of  $10^{-4}$  caused death in mice.

In view of the small number of culture generations and the absence of confirmation *in vitro* the cultivation of this virus should be considered as temporarily successful.

*Chorioallantoic Preparations*—Burnet<sup>55a</sup> inoculated the virus of louping ill into the chorioallantoic membrane and found this membrane to be a more sensitive indicator of the presence of the virus than the mouse brain. In a short time the virus was present in the blood, and death of the embryo usually occurred five or six days after inoculation. Hepatic damage was marked.

#### LYMPHOGRANULOMA VENEREUM

*Tissue Cultures*—The evidence presented for propagation of the virus of lymphogranuloma venereum is not consistent, and there is definite disagreement in the conclusions drawn by various investigators.

Meyer and Anders<sup>56</sup> reported maintenance of the virus through 6 generations in flask preparations containing guinea pig kidney, testis and



25 per cent guinea pig serum in Tyrode's solution. There was a final dilution of the original inoculum of about 1:64,000,000, but they were unable to obtain positive results with the Frei test in man when the culture material was used instead of human pus. However, inoculation of cultures into guinea pig inguinal glands caused inflammatory changes in about 50 per cent of the cases. This work was repeated by Miyagawa and his collaborators<sup>57b</sup> with little or no success.

Tamura<sup>59</sup> inoculated diluted infected pus into test tubes which contained guinea pig liver or kidney in Tyrode's solution. He asserted that cloudiness developed in the supernatant fluid and that the cloudiness could be transferred to new tubes even after filtration of the culture through a Berkefeld N filter. On staining with eosin-Giemsa stain, the supernatant fluid showed "peculiar granules." Control tubes remained clear, but it should be noted that these tubes, instead of being inoculated with inactivated virus, received no inoculum of any kind. This investigator reported 24, 12 and 14 transfers. Fluid from the twenty-third subculture gave a skin reaction similar to a positive Frei reaction. When 1 cc. of the supernatant fluid was inoculated into the groin of a guinea pig, adenitis resulted in two to three days. The inflammation underwent spontaneous resolution, but subsequently, in three or four weeks, sensitivity of the skin was apparent. Here, again, repetition of the procedure by Miyagawa and his co-workers<sup>57c</sup> yielded no results. However, D'Aunoy, von Haam and Lichtenstein<sup>58</sup> reported partial confirmation through 3 culture passages. Another failure to demonstrate increased potency of the virus with a preparation similar to Tamura's was reported by Voet<sup>60</sup>.

Miyagawa, Mitamura, Yaoi, Ishii and Okanishi<sup>57b</sup> inoculated infected mouse or squirrel brain into large hanging drop preparations of adult mouse testicle, brain or spleen, guinea pig plasma and mouse spleen extract. Second culture generations produced skin reactions of varying degrees resembling those to the Frei test and caused infection in mice. These investigators were able to stain intracellular "granulocorpuscles" in cultured tissue, but no statement is made as to further culture passages.

Malamos<sup>55a</sup> reported formation of inclusion bodies in hanging drop cultures of adult rabbit cornea after forty-eight hours' incubation. The source of the virus was an infected mouse brain. After cultivation of the virus for forty-eight hours, the *in vitro* material was inoculated into mice and caused typical symptoms.<sup>c</sup>

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(c) Three strains of the lymphogranuloma virus have been maintained by Sanders and Geish (unpublished data) in preparations containing embryonic guinea pig brain and ultrafiltrate of ox serum. Increased potencies of 1,000 times have been demonstrated, and consistent results have been obtained with animal inoculations and skin tests.

*Chorioallantoic Preparations*—Miyagawa and his co-workers<sup>57a</sup> were able to transmit the virus to mice after 5 egg passages and were also able to demonstrate the presence of "granulocorpuscles" in the brains of these mice. Nauck and Malamos<sup>58</sup> transmitted the disease to mice after 3 and 6 egg passages. According to these investigators, the membrane was lightly affected by single large spots. Unlike the previous workers, they were unable to demonstrate inclusions in the egg preparations.

In view of the lack of agreement among investigators of this virus, the small number of generations reported in most cases and the lack of a demonstrable increase in potency, it appears that the virus of venereal lymphogranuloma has been, at best, cultivated temporarily.

#### NEWCASTLE DISEASE

*Tissue Cultures*—Topacio<sup>61</sup> cultivated a strain of avian pest virus for 31 culture generations over a period of one hundred and twelve days, in Carrel flasks containing chick embryo tissue, Tyrode's solution and chicken plasma. Since each subculture was diluted 1:30 and since the final subculture was infective for chickens, there was no doubt of virus propagation. Recovered birds were found to be immune to doses of virus that normally were lethal, and subinfective doses of the cultivated virus could be given as a vaccine.

Aside from pyknosis of the nucleus, intracellular changes were not seen. There was no evidence of predilection for any tissue, the virus grew best on minced whole embryo.

*Chorioallantoic Preparations*—A study of two strains of this virus on the chorioallantois was made by Burnet and Ferry<sup>26</sup> in 1934. They found that the embryos were consistently killed within twenty-four to forty-eight hours. The membrane, extremely susceptible to the virus, was moist and edematous. Ectodermal proliferation and necrosis occurred, but the lesions were not sufficiently definite to permit titrations of potency by the counting method. Pathologic changes were not confined to the ectoderm. The mesoderm showed exudation, hemorrhage and edema. A characteristic effect of the virus on the embryo was the production of small, circumscribed hemorrhages in developing feather follicles. If small amounts of immune serum were used to retard the virus action, the embryo survived somewhat longer, and the lesions of the membrane were smaller and more localized.

#### PACHECO'S PARROT DISEASE

*Chorioallantoic Preparations*—Rivers and Schwenker<sup>62</sup> passed the virus of Pacheco's parrot disease in the chorioallantois for 6 generations. In a preparation incubated three days they noted opaque spots at the point of inoculation and distant discrete foci. Embryonic death occurred

usually from the third to the fifth day after inoculation. Occasional white spots were present in the liver, kidneys and spleen.

Microscopic examination of the infected membrane revealed ectodermal proliferation and necrosis. There was heavy inflammatory infiltration into the mesoderm, with inclusion bodies in both ectodermal and mesodermal cells. When the liver, kidneys and spleen were affected, they showed necrosis of cells and inclusions.

The final egg generation was infective for budgerigars. Chicks 1 or 2 days old could be infected, but transmission from chick to chick was unsuccessful.

The pathologic changes appeared to be specific, but the number of egg passages reported was small, and there has been no confirmation of this work. Consequently the cultivation of this virus should be considered as only temporary.

#### POLIOMYELITIS

*Tissue Cultures*—Propagation of the virus of poliomyelitis through 16 culture generations in preparations containing embryonic chick brain, monkey serum and Tyrode's solution was claimed by Gildemeister.<sup>64</sup> Subcultures were made every three or four days, and on the sixteenth transfer inoculation of the culture into a monkey produced the disease. Estimates of increased potency were not made. Gildemeister's work was confirmed by Pauli,<sup>66</sup> with 6 subcultures, but Plotz<sup>67</sup> and Sabin and Olitsky<sup>68</sup> were unable to repeat it.

Sabin and Olitsky<sup>68</sup> passed the virus through various human tissues in Tyrode's solution. The brain, cord, lungs, liver, kidney and spleen from a 3 to 4 month old human embryo were stored in the refrigerator for nine days. During this time portions of 100 mg weight in 4.5 cc were incubated at 37 C in 50 cc Erlenmeyer flasks. Each flask was inoculated with 0.5 cc of filtrate from a 5 per cent poliomyelitis cord suspension. Titration of the original inoculum showed 50 minimal lethal doses. Subcultures were made every three days, and a single embryo was used for 3 culture transfers. In this series, only preparations which contained nerve tissue were capable of infecting monkeys. One cubic centimeter of material from the sixth culture generation when inoculated into a monkey produced flaccid paralysis, which could be reproduced in animal series by using Berkefeld N filtrates. Histologic examination and serologic and other animal tests proved the infection to be poliomyelitis.

Attempts of these authors to cultivate the virus in chick embryo brain failed until a third culture transfer from human tissue was passed into chick brain. The virus was then maintained for 3 more generations. The authors were also unable to pass the virus in monkey cord tissue and mouse embryonic brain.

The opinion was expressed by Plotz <sup>67</sup> that the virus of poliomyelitis had been only maintained in tissue cultures, probably without real propagation. When one considers the conflicting results of work with chick embryonic tissues and the small number of culture passages in human tissues with no subsequent confirmation, such an opinion appears to be justified.

#### PSEUDORABIES

*Tissue Cultures*—In 1933 Traub <sup>68</sup> reported successful transmission of the virus of pseudorabies through 49 subcultures on chick embryonic tissue in Tyrode's solution. The final transfer was infective for mice on intraperitoneal injection. At this time it was noted that the disease produced with cultured virus had a shorter period of incubation, ran a more rapid course and was associated with larger lesions at the site of inoculation than the disease due to animal passage virus. Intranuclear inclusions were found in cultures.

The same author <sup>69</sup> later reported 6 passages of the virus in cultures made of immune guinea pig testis, Tyrode's solution and homologous serum. Dilution of the original inoculum was definitely apparent, but no estimate of potency was made.

*Chorioallantoic Preparations*—The virus was passed through 6 egg membranes by Mesrobianu <sup>70</sup> and through 57 egg membranes by Buinet, Lush and Jackson <sup>70</sup>. The former author found no change in virulence for animals, but the latter group noted decreased virulence for rabbits and mice.

#### PSITTACOSIS

*Tissue Cultures*—In 1932 Bedson and Bland <sup>71</sup> first reported cultivation of the virus of psittacosis in hanging drop preparations of mouse serum, salt solution (or chick embryonic extract) and mouse spleen. When normal spleen was used it was allowed to stand for an hour in the refrigerator in the presence of a fresh psittacosis filtrate. Otherwise, infected spleen tissue was used as the explant. After seventy-two hours of incubation at 37 C a thousandfold increase in potency was noted. When the cultured tissues were stained with Giemsa or with a modified Castaneda stain (solution of formaldehyde and Boriel blue with safranin counterstain), intracellular inclusions were found.

This work was repeated by Bland and Canti, <sup>72</sup> who used, in addition to mouse spleen and minced chick embryo, chick embryo lung, leg muscle and skin. They studied the cycle of development of inclusion bodies and found fibroblasts from leg muscle and cutaneous epithelium best suited for such work. Cultures were stained (Giemsa), examined by dark field illumination and by transmitted light, and photographed by means of the microcinematograph. Extracellular formation of inclusions was not seen. During the first eight hours elementary bodies appeared

During the next forty hours large homogeneous masses developed and the smaller elementary bodies decreased. By the end of seventy-two hours the small forms were not visible.

Successful propagation of this virus in chick embryo tissue (Levinthal<sup>76</sup>) and in embryonic chick liver (Haagen and Cidel<sup>75</sup>) has also been reported. During the course of his work with embryonic chick tissue Levinthal noted a definite predilection of the psittacosis virus for endothelial and epithelial cells.

The marked increase in potency, the extensive observations of inclusion formation *in vitro* and the consistent confirmation leave little doubt that this virus has been propagated.

*Chorioallantoic Preparations*—Chorioallantoic inoculation with the virus of psittacosis was easily done in 1935 by Fortner and Pfaffenberg<sup>74</sup> and by Burnet and Rountree<sup>73</sup>. According to the latter, numerous circumscribed conical lesions appeared on the thickened membrane three days after incubation. Histologic examination of the inclusion bodies gave results in agreement with the descriptions published by Bland and Cant<sup>1</sup>. At the height of infection in the membrane specific changes were found in the surface cells. Beneath these cells was a necrotic layer, which contained many leukocytes and which covered a rather thick stratum of uninfected proliferated ectoderm. The top ectodermal cells were flattened and cornified and served to limit the infectious process, so that when the necrotic cells were sloughed, the process did not extend to the embryo.

#### RABBIT FIBROMA

*Tissue Cultures*—Faulkner and Andrewes<sup>77</sup> passed an inflammatory strain through 10 culture generations in flask cultures and hanging drop preparations. Rabbit testicular tissue, normal rabbit serum (or plasma) and Tyrode's solution were used. Spleen extract was added to the hanging drops. The titer, originally  $1 \times 10^3$ , was increased by the eighth subculture to at least  $1 \times 10^9$ . Inclusion bodies were not seen in the cultures.

The same investigators were unable to maintain a fibromatous strain of this virus and noted that the gradual decrease in potency corresponded with degeneration of the cultivated tissues.

In view of the small number of culture passages, confirmation of this work is desirable before propagation of the virus of rabbit fibroma (inflammatory strain) is credited.

#### RABBIT MYXOMA

*Tissue Cultures*—The virus of rabbit myxoma has been successfully maintained for 20 subcultures in flask preparations containing mononuclear cells (Benjamin and Rivers<sup>78</sup>, Plotz<sup>82</sup>) and for 30 passages in

hanging drop cultures of rabbit testicular cells (Haagen<sup>60</sup>) Kidney, lung, white blood cells and lymph gland tissues have also been used with some success (Haagen<sup>60</sup>)

Actual propagation of the virus has been demonstrated by an increase in potency of 100 times after incubation in culture, and of 10,000 times after further transfers (Benjamin and Rivers) Plotz observed an increase of potency of 40 000 times by the seventeenth subculture and Haagen, a dilution activity of  $1 \times 10^9$  The latter investigator also noted that increase in virulence was much more marked when normal cells were added to the virus-containing tissue Plotz demonstrated that the virus did not survive more than fourteen days without transfer

Although confirmation has not been extensive, the consistent results obtained by the three groups of investigators, the moderately high number of culture passages and the marked increases in potency justify grouping this virus with those propagated in vitro

*Chorioallantoic Preparations*—The virus of myxoma has been cultivated on egg membranes by Lush<sup>61</sup> for 26 passages and by Haagen and Dscheng-Hsing<sup>62</sup> for 20 egg generations Although both investigators are agreed that this virus grows readily on the chorioallantois, they differ as to the pathologic changes produced and as to the virulence

According to Lush, incubation at 36 C was more effective than the usual incubation at 39 to 40 C He noted that ectodermal proliferation was the principal change in the membrane, that the embryo suffered no damage and that no inclusion bodies were present in the membrane or in the embryo Haagen, on the other hand, found, in addition to proliferative changes hemorrhages and cellular infiltration, with inflammatory changes in the mesoderm and entoderm By the third day death of the embryo occurred, and in later egg passages the life expectancy of the embryo was reduced to two days As a final point of difference Haagen claimed to have found inclusion bodies in epithelial and connective tissue cells

Since both Haagen and Lush experienced little difficulty in growing the virus on the chorioallantois, the difference in findings might well be accounted for by variation in the potency of strains In this respect it is interesting to note that Lush found no difference in the character of the virus after passage, while Haagen observed an increase in virulence However, since neither series was very long, such a discrepancy appears unimportant The fact remains that growth of the virus occurred in egg preparations

#### RABIES

*Tissue Cultures*—The first demonstration of the maintenance of fixed virus in nerve tissues of monkeys was that by Levaditi<sup>67</sup> in 1914 Although six to thirty-seven day cultures produced rabies in 5 of 6

monkeys, Levaditi felt that conservation of virulence rather than propagation of the virus had occurred. Later Stoel<sup>89</sup> passed infected rabbit or mouse brain through preparations containing either chick or rabbit skin or Rous sarcoma or mouse carcinoma for 4 culture transfers. However, no quantitative estimates of increase in potency were made, and the small number of transfers precluded definite evidence of virus growth.

More positive evidence of propagation was reported in preparations of embryonic rabbit (Kanazawa<sup>86</sup>), embryonic mouse (Webster and Clow<sup>92a,b</sup>, Bernkopf and Kligler<sup>84</sup>, Schultz and Williams<sup>88</sup>), embryonic mouse brain (Bernkopf and Kligler<sup>84</sup>) and embryonic chick tissue (Webster and Clow<sup>92b</sup>).

The number of successful culture passages has varied from 7 (Bernkopf and Kligler) and 10 (Kanazawa) to 16 (Webster and Clow, Schultz and Williams) and 42 (Webster and Clow). The titers of infectivity of various subcultures have not exceeded 1:1,000 (Kanazawa, Webster and Clow).

In attempting to use culture virus as an immunizing agent, Webster and Clow<sup>92b</sup> noted that mice receiving one intraperitoneal injection of the material were protected against 100 cerebral doses of street virus. Subcutaneous vaccination was not effective. Dogs were also immunized by use of the culture virus.

Although the number of culture transfers of rabies virus has not been high, consistent confirmation of the work and the relative ease with which increases in potency have been obtained justify placing this virus with those that have been propagated.

*Chorioallantoic Preparations*—Attempts to propagate the virus of rabies on the chorioallantoic membrane were unsuccessful previous to 1939. Waldhecker<sup>91</sup> reported negative results after 9 attempts with various strains. Schultz and Williams<sup>88</sup> experienced similar lack of success in 12 attempts to inoculate the egg membrane.

Dawson<sup>85</sup> recently reported successful passage of the virus of rabies through 12 egg generations. The virus was originally present in the brain of a rabid dog and was passed through a mouse. Both the dog and the mouse brain contained Negri bodies. Egg to egg transfer was done every six or seven days with the exception of the first egg generation, which was passed after four days. An emulsion of embryonic brain was used as passage material and was inoculated directly into embryonic brain for the next generation. Aside from the death of several embryos between the sixth and the eighth day after inoculation, there was no clinical evidence of rabies. As a rule, the embryos appeared to be healthy and, in the opinion of this investigator, would have hatched if they had not been put to death. Microscopic examination of embryos six and seven days after inoculation revealed many Negri bodies and acute neuronal necrosis.

When virus was inoculated into the brain, eye and thigh, on the chorioallantois and into the amnion of separate embryos, respectively, only the embryos inoculated in the brain and eye showed evidence of virus proliferation and the presence of Negri bodies. In 2 cases in which the virus was inoculated on the chorioallantois, the embryos were allowed to hatch and were apparently healthy.

The successful results noted by Dawson are particularly interesting in view of past failures to transmit this virus through egg preparations. The complete lack of response on the part of the chick embryo appears to have been due to choice of an unsatisfactory route of inoculation rather than to inability of the embryo to provide receptive cells for the virus.

#### RIFT VALLEY FEVER

*Tissue Cultures*—Mackenzie<sup>93</sup> inoculated material from the heart or liver of an infected mouse into chick embryonic tissue in Tyrode's solution and was successful in transmitting the virus of Rift Valley fever through 13 culture generations without loss of virulence. Dilution of the original inoculum was estimated at  $1:15 \times 10^{18}$ . Saddington<sup>94</sup> repeated this work and reported a series of 12 subcultures, the final culture of which was virulent for mice.

*Chorioallantoic Preparations*—Saddington<sup>94</sup> transferred the virus through 5 eggs and noted constant virulence for the embryos. Inflammatory and necrotic changes were seen in the membranes and in the livers.

#### ROUS SARCOMA

*Tissue Cultures*—In 1926 Carrel<sup>95d</sup> stated that the Rous sarcoma could be grown at will in cultures of chicken leukocytes in small quantities of plasma, Tyrode's solution and embryonic extract. Subcultures were made every two to three weeks. When pure strains of normal fibroblasts were grown in the presence of the virus, the cultures were not capable of producing tumors in chickens. This finding was consistent with previous reports by the same author that filtered extracts of Rous sarcoma deteriorated in the presence of fibroblasts<sup>95b</sup> and multiplied readily in the presence of leukocytes<sup>95a</sup>.

Further substantiation of the predilection of this virus for white blood cells was reported by Carrel and Ebeling<sup>95e</sup> while they were investigating the changes involved in the in vitro formation of fibroblasts from monocytes. They noticed that fibroblast formation in tissue cultures was hastened on addition of Rous virus to white blood cells. The resulting cells appeared to be "resistant" or "immune" and were unable to produce tumors.

Carrel<sup>95d</sup> also inoculated sarcoma filtrates into cultures in which the cellular components were in various stages of proliferation, viability



and death. He found that the virus could survive in the presence of dead cells but could multiply only when cellular proliferation occurred.

*Chorioallantoic Preparations*—Keogh<sup>96</sup> reported in 1938 that the virus of Rous sarcoma could be transmitted easily in chorioallantoic preparations. At the time of this report 30 egg passages had been done. Infections obtained with dilutions of  $10^{-4}$  in doses of 0.05 cc. in the later passages were good evidence of increased potency. Filtrates of the chorioallantois obtained with graded collodion membranes produced characteristic lesions in fowls. Titration of the virus on the membrane was done by inoculating dilute filtrates, which produced localized circumscribed lesions.

Usually no changes appeared in the membrane for three days after inoculation. A pathologic condition became apparent about the seventh day, and in Keogh's opinion, was typical of Rous sarcoma. The pathologic change was at first limited to the ectoderm except for slight inflammatory changes in the mesoderm. In addition to large central tumors, small, discrete, thickened, pearly lesions in the ectoderm were noted, which on microscopic examination revealed marked proliferation of this layer. As extension of the lesions took place, cells proliferated not only in lateral directions but also downward into the mesoderm. Peripheral cells stained in a manner suggestive of cornification. The cells which invaded the mesoderm were anaplastic, with large, homogeneously stained nuclei and an abundance of mitotic figures.

The results reported by Keogh are particularly interesting in view of their apparent contradiction of the extensive investigations of Carrel. It must be remembered that in the egg the principal pathologic alteration is initiated by ectodermal cells, and even extension of the lesions seems to be ectodermal. The conclusiveness of the results leaves little doubt that Rous sarcoma can be propagated both in tissue culture and on the chorioallantois. It is probable that future investigations will explain what appears to be an inconsistency at present, namely, why fibroblasts seem to exert a more baneful influence on the virus in tissue cultures than in egg preparations.

#### RUBEOLA (MEASLES)

*Chorioallantoic Preparations*—Magarinos Torres and de Castro Teixeira<sup>97</sup> reported inconclusive results with the chorioallantoic preparations. Egg membranes were inoculated with vesicle contents. The only pathologic change which they noted was localized proliferation of ectodermal cells, appearing as pale pinhead-sized nodules about the point of inoculation. Five passages were reported.

Much more definite evidence for virus propagation was presented by Wenkebach and Kunert,<sup>98</sup> who inoculated on the chorioallantoic membrane 5 cc. of blood taken from patients in the prodromal stages

of the disease. Virulence was tested by inoculating 0.5 cc of convalescent serum into eggs containing the virus and previously incubated for varying periods of time. As controls, normal adult serum and convalescent poliomyelitis, varicella and herpes simplex serums were used. Neutralization occurred only with the measles convalescent serum. Lesions could not be transmitted from egg to egg after addition of the specific serum and when infected blood came in contact with the chorio-allantois there appeared small opaque whitish spots, which varied in size from that of a pinhead to that of a lentil seed. In one series the lesions were transmitted through 13 egg passages, for a period of one hundred and fifty-six days. Thirteen strains were tried, and the authors contended that any series could be maintained at will. In 1 instance the virus after the fifth egg passage produced lesions at a dilution of 1:60,000.

The method used by Wenkebach and Kunert suggests a possible solution for the problem of testing viruses which are not transmissible to experimental hosts (e.g., varicella). In the case of measles further confirmation is desirable before propagation of the virus is considered definite.

#### SHEEP POX

*Tissue Cultures*—Bridé<sup>99</sup> inoculated flasks containing sheep testicular cells, serum and Drew's solution with fresh material. The fourth subculture was active at a dilution of 1:10,000 and produced typical lesions in sheep. No growth of the virus occurred after fourteen days.

In view of the small number of subcultures, the quick loss of virus and the lack of confirmation, the propagation of sheep pox virus in tissue culture should be regarded as questionable.

#### TRACHOMA

*Tissue Culture*—In 1937 Harrison and Julianelle<sup>101</sup> reported uniformly negative results in an impressive study of the virus of trachoma, a study which included many types of tissue cultures. Inoculums explanted tissues and mediums were varied in more than 1,100 tissue cultures but, according to the authors, the virus did not even survive, although its presence before inoculation had been frequently proved by animal infection. No inclusion bodies were found. Similarly Busacca<sup>100</sup> and Thygeson<sup>103</sup> were unable to demonstrate propagation of the trachoma virus.

Poleff,<sup>102</sup> however, asserted that he was successful in passing the virus through 5 subcultures in a modified Maitland medium described by Nigg and Landsteiner<sup>157</sup>. He also maintained that within two to three days there was a characteristic membrane formation of cells in culture and that inclusion bodies were present and were transmissible.

to the rabbit by intraocular inoculation. A rabbit infected in this manner was used as a further source of infection for 2 other animals. No mention was made of increase in potency.

In view of the negative results reported by the majority of workers and the small number of passages noted in the single claim to success, trachoma should be considered as not having been grown *in vitro*.

*Chorioallantoic Preparations*—Harrison and Julianelle<sup>101</sup> and Thygeson<sup>103</sup> observed no propagation when egg membranes were inoculated with trachoma.

#### VARICELLA (CHICKENPOX)

*Chorioallantoic Preparations*—De Castro Teixeira<sup>104</sup> reported 11 egg passages of lesions which originally had been caused by inoculation of the chorioallantois with the contents of a varicella vesicle. Histologically, the lesions were yellowish irregular nodules with adjacent areas of blanching. Other lesions, approximately the size of a pinhead, were disseminated at points distant from the site of inoculation. Titration of potency was not done and confirmation has not been forthcoming.

#### VARIOLA-VACCINIA

*Tissue Culture*—In 1906 Aldershoff and Boers<sup>105</sup> were the first to report successful maintenance of vaccinia virus outside the living body. Placing infected corneal tissue in a sterile "humid chamber" and apparently unaware of the significance of new cells, they stressed only the fact that Guarnieri bodies appeared after twenty hours. The experiment was repeated with hanging drop preparations of rabbit or guinea pig corneal tissue by Steinhardt, Israeli and Lambert<sup>135a</sup> in 1913 and by Steinhardt and Lambert<sup>135b</sup> in 1914. This time, notice was taken of the role of living cells and of the increase in potency of the virus from 6 to 10 times. In the following year Haide,<sup>115</sup> using rabbit and guinea pig testicular cells in a similar preparation, obtained an increase in viral potency.

Since these first successes numerous investigators have reported propagation of vaccinia virus in tissue cultures of all types. The virus appears to grow well in the presence of many tissues. Good results have been obtained with rabbit testicle (Plotz<sup>131a</sup>, Parker and Nye<sup>129b</sup>, Mervin and Schmerling<sup>125</sup>), corneal tissue (Craciun and Oppenheimer<sup>111b c</sup>, Rivers, Haagen and Muckenfuss<sup>132c</sup>), embryonic chick (Carrel and Rivers<sup>109</sup>, Li and Rivers<sup>121</sup>, Rivers<sup>132a</sup>, Rivers and Ward<sup>132g</sup>), chicken kidney (Maitland and Maitland<sup>121c</sup>, Rivers, Haagen and Muckenfuss<sup>132b</sup>) and rabbit kidney (Muckenfuss and Rivers<sup>126</sup>, Haagen<sup>114b</sup>).

It is apparent at once that the tissues mentioned in the foregoing paragraph have one factor in common, i. e. they are all rich in epi-

thelium, a finding consistent with the specific cellular predilection which vaccinia has shown in the past. Among others, Ciacini and Oppenheimer<sup>111b</sup> and Nauck and Robinow<sup>127d</sup> have cultivated the virus in pure epithelial cultures. Haagen<sup>114a</sup> went a step further and tested the affinity of the virus for various tissues. He ascertained that it could be maintained for only several culture generations if fibroblastic tissue predominated in the preparations and that epithelial and endothelial cells were necessary for growth of the virus.

Propagation of the virus has been shown to occur *in vitro* when the cells were viable but not proliferating (Rivers, Haagen and Muckenfuss<sup>13-b</sup>, Plotz<sup>131b</sup>), as well as in the presence of living, actively proliferating cells (Ciacini and Oppenheimer<sup>111b,c</sup>, Steinhardt, Israeli and Lambert<sup>135a</sup>, Williams and Flouinoy<sup>137</sup>, Hirano<sup>117</sup>). The extensive tolerance of the virus of vaccinia for variations of cellular metabolism is emphasized by the important contribution of Li and Rivers,<sup>121</sup> who succeeded in growing the virus in a preparation of chick embryo tissue and Tyrode's solution. The cultivation of a virus was thus reduced to a matter of the simplest of *in vitro* preparations—living cells plus a balanced salt solution. This work was later confirmed by Rivers,<sup>132a</sup> Rivers and Ward<sup>132g</sup> and Plotz<sup>131b</sup>), who respectively succeeded in passing vaccinia strains through 15, 90 and 150 culture passages.

The growth requirements of vaccinia virus were further studied by Maitland, Laing and Lyth,<sup>121b</sup> who found a correlation between the surviving respiratory activity of tissues and their suitability for promoting growth. Testis, kidney, liver and spleen, in descending order were effective for respiratory function. Moreover, growth occurred to a much better degree in the wide, well aerated Carrel flask than in a narrow test tube.

Increased potency of this virus in tissue cultures has been frequently demonstrated. As techniques have been improved and data concerning growth requirements accumulated, the increases in potency have attained higher values. Thus, in 1914 Steinhardt and Lambert<sup>135b</sup> observed an increase in potency of 6 to 10 times, while in 1925 Parker and Nye<sup>129b</sup> reported a multiplication of virulence of 51,000 times in the eleventh generation, and Maitland and Maitland<sup>124c</sup> in 1928, an increase in virulence of  $25 \times 10^6$ . Other high increases were noted by Carrel and Rivers<sup>109</sup> of 40 to 400 times after eight days of cultivation and by Muckenfuss and Rivers<sup>120</sup> of 10 to 1,000 times after each culture generation.

Confirmation of the presence of inclusion bodies in *in vitro* cultivation of vaccinia has not been lacking. In addition to reports by earlier workers (Aldershoff and Boers<sup>107</sup>, Parker and Nye<sup>129b</sup>), Rivers, Haagen and Muckenfuss,<sup>122c</sup> Rivers and Ward<sup>132g</sup> and Haagen<sup>114b</sup>

reported the presence of these bodies in cultures. According to Plotz,<sup>131b</sup> plasma and proliferating cells are important factors in the production of inclusion bodies.

Investigations of immune reactions in vaccinia tissue cultures have yielded somewhat conflicting results. Preparations containing plasma and corneal tissue of immune animals, or normal tissues to which immune serum had been added, did not support growth of the virus (Steinhardt and Lambert<sup>135b</sup>, Nye and Parker<sup>128</sup>). However, according to Rivers and Waid,<sup>132g</sup> vaccinia virus survived at least forty-eight hours in cultures of immune cornea in normal plasma and to a lesser extent in cultures of immune cornea in immune serum. When normal cornea was inoculated *in vitro* with virus and then cultivated in antivaccinia plasma, typical lesions with Guarnieri bodies occurred in twenty-four to forty-eight hours. If, however, immune cornea was used in normal or antivaccinia plasma, a mild reaction or no reaction at all occurred. As a result of these findings, Rivers and Waid hypothesized that once virus has combined with the cell, it cannot be neutralized by addition of immune substances. This was borne out by Rivers, Haagen and Muckenfuss,<sup>132d</sup> who observed that immune corneas inoculated and cultivated in immune plasma showed very mild infection with only occasional Guarnieri bodies.

It is not surprising that results of such investigations are not always consistent with immune reactions in the living body, since one is dealing with an isolated simple system and not with a coordinated complex organism, in which multiple immune mechanisms are at work. An example of the caution necessary in applying *in vitro* manifestations to *in vivo* conditions may be seen in the work of Beard and Rous<sup>107</sup>. These authors noted that the activity of vaccinia virus was reduced when a mixture of the virus and Kupffer cells was allowed to stand for a few minutes before intradermal injection. This apparently confirmed Ledingham's observation that in skin into which India ink had been injected there was no reaction to the virus, owing, he thought, to mobilization of Kupffer cells in response to the ink. However, when Beard and Rous cultivated the virus of vaccinia with Kupffer cells *in vitro*, the virus proliferated very well during the first six or seven days. It seems, as was suggested by the authors, that some condition present *in vivo* allowed an antiviral substance to be present in Kupffer cells.

The logical outgrowth of *in vitro* cultivation of the virus of vaccinia was its use in Jennerian prophylaxis. In 1931 Rivers,<sup>132a</sup> using a vaccine of tissue culture virus, successfully vaccinated 3 infants, and in the following year 7 positive reactions were similarly reported by Herzberg<sup>116a</sup>. In 1933 Rivers and Ward<sup>132g</sup> reported 100 typical lesions obtained in 118 vaccinations.

Less favorable were the results of Plotz and Martin<sup>131c</sup> Of 38 children vaccinated with lymph and with culture vaccines, the lymph produced typical lesions in all, while the culture vaccine gave only 19 positive reactions. However, in another series, better results for the culture vaccine were obtained by doubling the dose.

The advocates of culture vaccine claim that it is free from bacterial contamination and may be used in the fresh state (green calf lymph may not) and that it is cheaper and more convenient. On the other hand, the opponents maintain that the percentage of positive reactions obtained with culture vaccine is smaller than with calf lymph, that the use of culture vaccine is not a time-tried method, that in long-continued tissue cultures the virus is attenuated and that larger areas of the skin must be scarified. Rivers and Ward<sup>132b</sup> showed that although the titer of the cultures went down from  $10^{-6}$  in the nineteenth generation to  $10^{-1}$  in the eighty-sixth generation, potency was easily reestablished to  $10^{-6}$  by passage through 3 rabbits (intratesticular inoculation). Furthermore, the reestablished titer of  $10^{-6}$  was retained for 60 culture generations without further animal passage. However, recent work by Rivers, Ward and Baird<sup>1321</sup> with this "revived" strain has placed certain qualifications on their earlier contentions. Although they reiterated confidence in the use of culture vaccine, they felt that after extensive subcultures a qualitative change occurred in the virus which permitted large intradermal inoculations without damage. In their opinion, the immunity conferred by this strain was not completely protective, and revaccination within six months to one year with a potent calf lymph virus would produce the desired protection without untoward reactions. From the new evidence available, it seems that attenuation of culture virus occurs and that tissue culture vaccine may today be best used as an adjunct to lymph vaccine.

*Chorioallantoic Preparations*—The virus of vaccinia was first propagated on the chorioallantoic membrane in 1932, by Goodpasture, Woodruff and Buddingh<sup>113a</sup>. Confirmation has been ample and frequent, so that the macroscopic and microscopic changes are now well known. Although the dermal and neurotropic strains produce somewhat different lesions, they can both be cultivated with comparative ease on the chorioallantois. Goodpasture, Buddingh, Richardson and Anderson<sup>113b</sup> reported that each strain maintained the ability to produce specific lesions on the egg membrane even after many egg passages.

According to Burnet,<sup>108</sup> who has summarized this subject up to 1936, the early gross changes produced by both strains appear as small milky opacities on the membrane. With the dermal strain the first change is apparent in twenty-four hours. At forty-eight hours the lesions are enlarged (1 to 2 mm in diameter) and discrete, with an occasional

central depression. At seventy-two hours the lesions are still larger, and new ones appear. In the case of the neurotropic strain it is forty-eight hours before the first gross changes are visible. With time there occur necrosis and hemorrhage in the central portion of the lesions and also conspicuous central depressions.

Although Burnet stated that vaccinal strains are not very virulent for the embryo, death, when it occurs, usually takes place after the fourth day and is more likely to be produced by the neurotropic strain. Stevenson and Butler,<sup>136</sup> on the other hand, reported that they had never seen an embryo affected by this virus.

When the inoculum is massive or is concentrated, coalescent lesions occur which reveal the characteristic pathologic processes of the individual strains. In general, the lesion caused by the dermal strain tends to be proliferative, while that caused by the neurotropic strain is characterized by hemorrhage, thrombosis of vessels, edema and exudation.

Histologically, ectodermal proliferation or necrosis may occur, depending on the strain. Mesodermal inflammatory changes and proliferation of vascular endothelium are to be seen.

While Burnet<sup>108</sup> rarely noted Guarnieri bodies, inclusion bodies in egg preparations have been reported by many others (Goodpasture, Woodruff and Buddingh<sup>113a</sup>, Herzberg<sup>116c</sup>). Goodpasture, Buddingh, Richardson and Anderson<sup>113b</sup> not only observed inclusions but also noted that Paschen corpuscles diminished as infectivity decreased.

Jennerian prophylaxis with egg preparations has been successful in the hands of Goodpasture and his co-workers, Burnet, Herzberg, Stevenson and Butler, Lehman and others.

It is not certain whether the potency of vaccinal virus is decreased after long passage through eggs. Kunert<sup>120</sup> found a marked decrease in potency after 50 egg passages. Lehman,<sup>122a</sup> however, reported that virulence for rabbits increased with egg passage. This same author<sup>122b</sup> in 1937 used vaccine from the ninety-fourth egg passage (three years) with good results. In 2,500 revaccinations he obtained 95.8 per cent positive reactions. In 2,552 primary vaccinations he obtained 80.6 per cent positive results. Marked pustule formation without any complications was the rule in this series. Goodpasture, Buddingh, Richardson and Anderson<sup>113b</sup> vaccinated 1,074 persons from 4 to 20 years of age with a vaccine prepared after the hundredth egg passage and in the 978 patients receiving vaccination for the first time observed 93.6 per cent positive reactions. The reactions obtained were of the immune type.

In the opinion of Goodpasture and his collaborators, vaccine prepared from the chorioallantoic membrane is preferable to tissue culture vaccine because of its ability to maintain high titers without animal passage, and because it appears to have less pathogenicity for man while at the same time conferring a strong degree of immunity.

## VESICULAR STOMATITIS

*Tissue Cultures*—Cairnel, Olitsky and Long<sup>110</sup> inoculated the virus of vesicular stomatitis into flasks containing guinea pig embryonic tissue or bone marrow cells, plasma, Tyrode's solution and guinea pig or chick extract. When this preparation was incubated seven to ten days and titrated by guinea pig inoculation, an increase in potency of 10 to 1,000 times for each passage was noted. However, not each subculture was infective.

Cox, Syverton and Olitsky,<sup>111</sup> using filtrates of mouse brains infected with New Jersey and Indiana strains, cultivated these strains in preparations containing Tyrode's solution and minced chick embryos from which the heads and limbs had been removed. Fifteen culture generations for each strain were reported, with an increase in potency of  $10^6$ . Later the authors<sup>112</sup> repeated this work and obtained 35 and 58 culture generations with respective increases in potency of  $10^{36}$  and  $10^{61}$ .

*Chorioallantoic Preparations*—Burnet and Galloway<sup>139</sup> passed the two strains of this virus through 10 and 15 egg passages. The Indiana strain appeared to be more lethal for the embryo, although not regularly so. Histologic examination showed ectodermal proliferation with ultimate degeneration and necrosis of cells. Edema and congestion were marked, and death of the embryo was frequent. They found the virus present in the egg membrane and in the skin and liver of the embryo. Suspensions of the membrane proved infective in dilutions of 1:10,000. However, nonlethal strains, which produced lesions that were localized to the membranes, were observed. These authors noted that the egg preparations were much more sensitive to the higher dilutions than guinea pigs, and they were able to test immune serums with the chorioallantoic technique.

## VIRUS III

*Tissue Culture*—Andrewes<sup>142a</sup> reported in 1929 that he was able to cultivate virus III with comparative ease in Cairnel flasks which contained normal and infected rabbit testicular cells, Tyrode's solution and rabbit plasma or serum. This series was passed through 10 generations with (in cases in which serum was used) an increased potency of  $3.2 \times 10^6$ . He found inclusion bodies occurring regularly in healthy cells. In another series Andrewes<sup>143b</sup> succeeded in passing the virus through 23 subcultures with an increase in potency of at least  $8 \times 10^{27}$  times. During this work he was unable to demonstrate inclusion bodies when the testicular cells were replaced by liver, spleen, kidney or bone marrow cells. With a preparation similar to that used by Andrewes, Topacio and Hyde<sup>145</sup> passed the virus through 8 subcultures and demonstrated a final dilution activity of 1:300,000,000. Ivanovics and Hyde<sup>144</sup> repeated the work done by previous investigators and were able to get propa-



gation of the virus in other rabbit tissues, as well as in testis, although the latter was the most receptive for virus III

Results vary as to the formation of inclusion bodies in the presence of immune tissues and serums. Andriewes<sup>143b</sup> found that as long as the virus came in contact with the living cells before the addition of immune substance to the culture the inclusion bodies were formed. Topacio and Hyde,<sup>145</sup> however, found that even if the virus was allowed to be in contact with the cells for a whole hour before the addition of the immune plasma the formation of inclusion bodies was inhibited. The inconsistency of immune reactions in vitro is interesting in view of the frequency with which it occurs. (See the section on variola-vaccinia.) It is apparent that either the techniques are not identical, as they are thought to be, or some factor or factors not now known are present which influence the receptivity of cells for virus.

#### YELLOW FEVER

*Tissue Culture*—Haagen and Theiler<sup>147d</sup> in 1932 successfully passed a strain of the virus of yellow fever through cultures of chick embryonic tissue in Tyrode's solution and 10 per cent monkey serum for 60 culture generations over a period of eight months. Before cultivation this strain had been modified by intracerebral passage through many mice generations. Later Haagen<sup>147a, c</sup> showed that living cells were necessary for growth of the virus and that it had a distinct predilection for epithelial cells.<sup>147b</sup> In another series he was able to pass the virus through 100 subcultures and to demonstrate its close affinity for the cells in culture as compared with the fluid components. During these experiments Haagen<sup>147c</sup> noted that practically no propagation of the virus occurred if the serum was removed from the culture preparations.

Lloyd, Theiler and Ricci<sup>149</sup> cultivated the pantropic and two types of the neurotropic strain in flasks containing serum and Tyrode's solution with either chick or mouse embryonic tissue or adult mouse or guinea pig testicular tissue. The pantropic strain was passed through more than 150 subcultures without intercurrent animal passage over a period of twenty-one months. Prolonged cultivation of this pathogenic strain caused a consistent progressive inability to produce yellow fever. Virulence for monkeys was regained only after 30 passages through animals.

Theiler and Smith<sup>152a</sup> found that definite modification of yellow fever strains occurred, depending on the nature of the tissue used in the medium. In the presence of chick embryonic tissue there was marked loss of both neurotropism and viscerotropism without loss of antigenicity. According to the same authors,<sup>152b</sup> a high concentration of virus was best insured by infecting whole chick embryos. By using a vaccine prepared from such infected whole chick embryo, they were

TABLE 1—Group 1 *Viruses Which Have Been Propagated in Tissue Cultures (All Criteria Have Been Met)*

Virus	Maximum Number of Subcultures	Maximum Increase in Potency	Inclusion Bodies in Vitro	Confirmation
Vaccinia	170 <sup>171b</sup>	25 × 10 <sup>6</sup> (170)	Regularly	++++
Yellow fever	227 <sup>140</sup>	Apparent but not estimated		++++
Fowl plague	250 <sup>141</sup>	1,000 times in 18 hours		++++
Psittacosis	At will	1,000 times in 72 hours (102)	Regularly	+++
Virus III	23 <sup>141b</sup>	8 × 10 <sup>-7</sup>	Regularly	+++
Herpes simplex	60 <sup>171a</sup>	1.4 × 10 <sup>-28</sup> (51)	Found (51) and confirmed (59)	+++
Rous sarcoma	At will <sup>171d</sup>	Apparent but not estimated		+++
Foot and mouth disease	62 <sup>171</sup>	4.8 × 10 <sup>10</sup> (32)		+++
Coryza	58 <sup>171c</sup>	1 10 <sup>-7</sup> (11)		++
Vesicular stomatitis	58 <sup>142</sup>	10 <sup>01</sup> (204)		++
Rabies	42 <sup>172b</sup>	Apparent by activity on dilution		++
Rabbit myxoma	70 <sup>171</sup>	1 × 10 <sup>10</sup> (111)		++
Encephalitis (St. Louis)	26 <sup>171a</sup>	Activity on dilution 10 <sup>-7</sup>		++
Pseudorabies	49 <sup>171a</sup>	Apparent	Found (100)	+
Influenza	70 <sup>171a</sup>	Apparent		+
Equine encephalomyelitis	73 <sup>142</sup>	10 <sup>-1</sup> (204)		+
Encephalitis (Japanese B)	10 <sup>12b</sup>	1st culture activity 1 10 <sup>6</sup>		+*
Rift Valley fever	13 <sup>171</sup>	Dilution of original inoculum 1 1.5 × 10		+*

\* Further confirmation is desirable

TABLE 2—Group 2 *Viruses the Propagation of Which in Tissue Cultures Has Been Doubtful or Temporary (One or More Criteria Have Not Been Met)*

Virus	Maximum Number of Subcultures	Maximum Increase in Potency	Inclusion Bodies in Vitro	Confirmation
Newcastle disease	31 <sup>171</sup>	Definite but not estimated		0
Kikuth's canary virus*	10 <sup>15-</sup>	Definite but not estimated	Found	0
Lymphogranuloma venereum	24 <sup>170a</sup>	Dilution of original inoculum, 1 64 × 10 <sup>6</sup>	(82, 84, 85) Found?	Markedly inconsistent
Avian pox (fowl pox)	12 <sup>1</sup>	20 times (decreasing potency)		+
Ectromelia	7 <sup>10a</sup>	Dilution of original inoculum, 6 × 10 <sup>14</sup>		Insufficient
Fowl leukosis	1 strain 153 days <sup>21a</sup>	Irregular but not high		Inconsistent
Hog cholera	15 <sup>142</sup>	Dilution of original inoculum 10 <sup>20</sup>		0
Louping ill	11 <sup>174</sup>	8th culture infectious 10 <sup>-4</sup>		0
Rabbit fibroma (inflammatory strain)	10 <sup>77</sup>	1 10 <sup>0</sup>		0
Sheep pox	4 <sup>170</sup>	Activity at 1 10,000		0
Poliomyelitis	16 <sup>171</sup>	Questionable		Inconsistent

\* This virus can probably be grown at will, but confirmation of the observation is necessary

able to produce immunity in monkeys as measured by the titers of antibodies that had developed. These titers had no relation to the amounts of virus inoculated. The authors were able also to increase the antibody content in the blood of immune persons by subcutaneous inoculation of the culture virus.

The results obtained by their use of vaccine prepared from tissue cultures of yellow fever virus represent the best application to date of in vitro methods to practical purposes. The vaccine has proved itself efficacious and convenient. The extent of its success is revealed by the following statistics in the 1937 annual report of the Rockefeller Foundation<sup>150</sup>. Between February 1937 and February 1938 more than 59,000 persons, mostly in Brazil, were vaccinated. Mild reactions, or none at all, occurred. More than 95 per cent of the subjects vaccinated in field

TABLE 3—Group 3 *Viruses the Propagation of Which in Tissue Cultures Has Been Unsuccessful*

Virus	Maximum Number of Subcultures	Maximum Increase in Potency	Inclusion Bodies in Vitro	Confirmation
Trachoma	5 <sup>102</sup>	0		0
Herpes zoster	1 explant, no transfers	0		0
Inclusion blennorrhea	0	0		0
Rabbit fibroma (fibromatous strain)	0	0		0

work showed immunity. In a series of 38,000 vaccinated persons 69 missed a day or more of work. Not a single serious reaction was recorded for this series of vaccinations. In 1938 (January to October) 800,000 persons were vaccinated in Rio de Janeiro, and no serious complications occurred.

*Chorioallantoic Preparations*—The virus of yellow fever was transmitted through chorioallantoic preparations for a short number of passages by Elmendorf and Smith<sup>146</sup> and Jadin<sup>148</sup>. Increased potency was apparent in the virulence of high dilutions of either the chorioallantois or the embryo. No variation in the character of the virus was noted.

The opinion expressed by Jadin that this virus can be propagated at will in eggs seems to be well founded in view of the observation made by Theiler and Smith<sup>152b</sup> that high concentrations of yellow fever virus were obtained by infecting whole chick embryos.

SUMMARY OF OBSERVATIONS ON THE PROPAGATION OF VIRUSES  
IN CHORIOALLANTOIC PREPARATIONS

- 1 Variola-vaccinia May be grown at will Inclusions present Confirmed
- 2 Avian pox (fowlpox) Grown at will (Woodruff and Goodpasture<sup>138</sup>)  
Increase in potency shown by count of lesions (Burnet<sup>108</sup>) Inclusions present  
(Woodruff and Goodpasture<sup>138</sup>, Burnet<sup>108</sup>) Confirmed
- 3 Psittacosis Consistent propagation Confirmed Inclusions present Specific pathologic changes
- 4 Infectious laryngotracheitis of fowls Grown and consistently confirmed  
by four investigators Definite pathologic changes Inclusions present
- 5 Yellow fever Small number of passages (Elmendorf and Smith<sup>146</sup>,  
Jadin<sup>148</sup>) but probably can be grown at will
- 6 Encephalitis (St Louis) 68 passages (Smith<sup>15</sup>) Confirmed
- 7 Encephalitis (Japanese B) 75 passages (Haagen and Crodel<sup>12b</sup>) One  
confirmation Possible inclusions
- 8 Equine encephalomyelitis Small number of passages but fairly well confirmed  
Inclusions present (Covell<sup>17</sup>)
- 9 Fowl plague Grown without difficulty (Burnet and Ferry<sup>26</sup>) but number  
of egg passages not stated No confirmation
- 10 Influenza Grown at will Well confirmed
- 11 Louping ill Fairly specific pathologic changes Confirmed (Burnet and  
Lush<sup>53b</sup>) Better results than with tissue culture
- 12 Rabbit myxoma 26 passages (Lush<sup>81</sup>) Disagreement as to pathologic  
changes and presence of inclusions (Lush<sup>81</sup>, Haagen and Dscheng-Hsing<sup>37</sup>)  
Further confirmation desirable
- 13 Vesicular stomatitis 15 passages (Burnet and Galloway<sup>139</sup>) Egg preparations  
more sensitive than guinea pigs Definite pathologic changes
- 14 Herpes simplex 24 and 50 passages (Saddington<sup>39</sup>, Burnet, Lush and  
Jackson<sup>30</sup>) Pathologic changes not altogether specific Probably can be grown  
at will Further confirmation desirable
- 15 Kikuth's canary virus 8 transfers (Burnet<sup>51a</sup>) Confirmed (Burnet and  
Lush<sup>51c</sup>) Inclusions present Pathologic process similar to poxes Can probably  
be grown at will
- 16 Newcastle disease Can probably be grown at will (Burnet and Ferry<sup>26</sup>)  
but this has not been confirmed
- 17 Rous sarcoma 30 passages (Keogh<sup>96</sup>) Probably can be grown at will  
Specific pathologic changes Confirmation desirable
- 18 Pseudorabies 57 passages (Burnet, Lush and Jackson<sup>30</sup>) Can probably  
be grown at will Further investigation desirable
- 19 Rabies 1 positive report (Dawson<sup>85</sup>) 12 passages Suggestive pathologic  
picture Inclusions present Confirmation desirable
- 20 Ectromelia 18 passages (Paschen<sup>11</sup>) Good confirmation Inclusions  
(Paschen<sup>11</sup>)
- 21 Infectious bronchitis of chicks 14 passages (Beaudette and Hudson<sup>44</sup>)  
No confirmation but good evidence for increased pathogenicity
- 22 Rubeola (measles) 13 passages (Wenkebach and Kunert<sup>98</sup>) Suggestive  
pathologic picture Good neutralization with specific serum Further confirmation  
desirable

- 23 Varicella 11 passages (de Castro Teixeira <sup>104</sup>) Pathologic changes do not appear to be very specific No evidence for increase No confirmation
- 24 Coryza 3 passages (Kneeland, Mills and Dochez <sup>7a</sup>) No confirmation
- 25 Rift Valley fever 5 passages (Saddington <sup>91</sup>) No confirmation Additional evidence desirable
- 26 Pacheco's parrot disease 6 passages No confirmation Fairly specific pathologic changes Inclusions present Confirmation desirable
- 27 Lymphogranuloma venereum Small number of passages Disagreement as to inclusions Considered not grown Pathologic effects not definite
- 28 Lymphocytic choriomeningitis <sup>59a</sup> 8 passages Definite increase in potency No confirmation
- 29 Herpes zoster 3 passages (de Castro Teixeira <sup>10</sup>) No confirmation Pathologic effects not specific Considered as not grown
- 30 Foot and mouth disease Did not survive for twenty-four hours in the egg (Galloway and Elford <sup>20</sup>)
- 31 Trachoma Negative results (Harrison and Julianelle <sup>101</sup>, Thygeson <sup>103</sup>)

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## Notes and News

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**University News, Promotions, Resignations, Appointments, Deaths, etc**—Cornelius P Rhoads, associate member of the Rockefeller Institute for Medical Research, has been chosen to succeed James Ewing, who is about to retire, as director of the Memorial Hospital for the Treatment of Cancer and Allied Diseases in New York. Under Dr Ewing's wisely progressive guidance the Memorial Hospital has developed into a great modern center for cancer service and study.

T D Spies, associate professor of medicine in the University of Cincinnati, has been awarded the John Phillips Memorial Medal by the American College of Physicians for his contributions to the study of nutrition and particularly for his study of pellagra.

Eugene M Landis, assistant professor of medicine in the University of Pennsylvania, has been appointed professor of internal medicine in the University of Virginia.

Laszlo Detre, senior immunologist in the division of infectious diseases of the National Institute of Health, Washington, D C, died on May 26 of brain tumor, aged 64. Dr Detre discovered independently a test for syphilis almost identical with the Wassermann test. He graduated from the University of Budapest in 1895.

The first degree of doctor of medical science in forensic medicine from New York University College of Medicine has been awarded to Maurice Powers, director of the laboratory of the Royal Canadian Mounted Police in Regina, Sask, Canada.

F C Minett, professor of pathology in the Royal Veterinary College, London, has been appointed director of the Imperial Veterinary Research Institute of the Government of India.

Gerald B Webb, director of the Foundation for Research in Tuberculosis at Colorado Springs, has received the Trudeau Medal of the National Tuberculosis Association in recognition of his attempts "to produce specific immunity against tuberculosis by the inoculation of animals with very minute numbers of tubercle bacilli."

The American Institute of Nutrition has awarded C A Elvehjem, professor of biochemistry in the University of Wisconsin, \$1,000 for his discovery that nicotinic acid will cure pellagra.

**Society News**—The American Association for the Advancement of Science will hold its next annual meeting at Columbus, Ohio, Dec 27-30, 1939.

# Abstracts from Current Literature

TO SAVE SPACE THE ORIGINAL TITLES OF ABSTRACTED ARTICLES SOMETIMES  
ARE SHORTENED

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## Experimental Pathology and Pathologic Physiology

GENESIS OF RENAL HYPERTENSION L N KATZ, M FRIEDMAN, S ROBBARD and  
W WEINSTEIN, *Am Heart J* **17** 334, 1939

The genesis of the hypertension following renal ischemia (Goldblatt method) was reinvestigated in an attempt to demonstrate a humoral mediator in hypertensive animals. The average femoral arterial blood pressure of 127 normal trained dogs, determined by the Hamilton technic, was 155 mm of mercury systolic and 80 mm diastolic. The average brachial arterial blood pressure of 16 normal trained dogs, determined indirectly, was 130 systolic and 85 diastolic. The hypertension which developed in unilaterally nephrectomized animals was more severe and of longer duration than that in animals which had two normal kidneys, 50 per cent of the nephrectomized animals died in uremia. Partial occlusion of both renal arteries was performed in 24 dogs. In 85 per cent of them hypertension developed, which was more severe and of longer duration than when partial occlusion of only one renal artery was produced. The nonprotein nitrogen of the blood usually became elevated following clamping, and 7 dogs died in uremia.

The severity and persistence of the effects of renal ischemia depend on the presence of a normal kidney. This indicates that hypertension depends on the ratio of ischemic to normal renal tissue. Distemper was found to cause alleviation or disappearance of renal hypertension, the hypertension recurred when the distemper was cured. Partial thyroparathyroidectomy did not affect the blood pressure in renal hypertension. Histologic examination of kidneys in which ischemia had been present as long as six months failed to reveal definite abnormalities.

Cross transfusion of several liters of whole blood for eighteen or more hours between trained unanesthetized dogs with persistent renal hypertension and trained unanesthetized, bilaterally nephrectomized, nonhypertensive dogs failed to reveal any pressor response in the nonhypertensive dogs. Perfusion of large quantities of heparinized blood over several hours from an anesthetized dog with persistent renal hypertension into the isolated denervated hindlimb preparation failed to produce any vasopressor action in the latter.

Temporary hypertension lasting several hours was found to follow unilateral or bilateral nephrectomy. This did not occur in control, mock operations. It is suggested that the transient hypertension following nephrectomy is neurogenic.

FROM AUTHORS' SUMMARY

EXPERIMENTAL THROMBOPENIC PURPURA L M TOCANTINS and H L STEWART,  
*Am J Path* **15** 1, 1939

The clinical and pathologic manifestations of experimental purpura as produced with antiplatelet serum in the dog undergo a uniform evolution, the stages of which may be conveniently divided into (1) an acute stage (first to fifth days), in which occur thrombopenia, with a prolonged bleeding time, and, in the tissues, hemorrhage, edema and deposition of pigment, (2) an intermediate stage (fifth to tenth days), during which there are a rising platelet count, a short bleeding time and multiple vascular thrombi in various organs, principally in the spleen, (3) a reactive stage (after the tenth day), characterized by a high platelet count and hyperplastic changes in the bone marrow, spleen, lymph nodes, thymus and Peyer's patches of the ileum. There are indications that these evolutionary changes of the experimental disease are duplicated to a certain extent in spontaneously occurring thrombopenic purpura of man.

FROM AUTHORS' SUMMARY

NEPHRITIS AND HEMOGLOBIN PRODUCTION IN ANEMIA G H WHIPPLE and  
F S ROBSCHT-ROBBINS, J Exper Med **69** 485, 1939

Spontaneous glomerulitis develops not infrequently (incidence, 11 per cent) in dogs of the anemia colony. The course of the nephritis is insidious and usually extends over several years but ends in uremia, often with terminal bronchopneumonia. The production of hemoglobin in these standard anemic dogs is well established as related to various standard food factors. Tables show the changes that appear year by year in the life of each dog. Nephritis causes little or no change in the production of hemoglobin in these anemic dogs in its early stages. In the late stages there may be no change or moderate changes in the production of hemoglobin. In advanced nephritis the average is 70 per cent of the normal production. It seems unlikely that this degree of impairment in the production of hemoglobin in nephritis would result in spontaneous anemia in the dog.

FROM AUTHORS' SUMMARY

THE VI ANTIGEN OF THE TYPHOID BACILLUS D W HENDERSON, Brit J Exper  
Path **20** 11, 1939

Henderson finds that the antiserum protects mice against typhoid infection more efficiently when the infecting dose is a small number of highly virulent organisms than when a large number of less virulent ones are injected. The efficiency of the protection is thus determined by the mass of bacterial antigen in the tissues rather than by the virulence of the strain used.

ACROMEGALY AND ARTERIAL TENSION M JEQUIER, Ann d'anat path **13** 179,  
1936

At autopsy on a 34 year old woman with acromegaly of the classic type there was found all the anatomic evidence of severe primary hypertension. The eosinophilic adenoma of the hypophysis in this case was composed of young, immature cells. A review of the reports of cases of acromegaly in the literature reveals that there has been no constant association with hypertension. Jequier claims, however, that he has not been able to find any record in the literature of a case resembling his exactly, i. e., one in which there was a pituitary adenoma composed of young, undifferentiated eosinophilic cells. The question of a possible relationship between the younger eosinophilic cells of the hypophysis and the control of arterial tension is suggested but remains unsettled.

PERRY J MELNICK

HISTOLOGIC STUDY OF THE PARATHYROIDS AFTER EXPERIMENTAL TOTAL THYROID-  
ECTOMY H WELTI, R HUGUENIN and L BARRAYA, Ann d'anat path **13**  
**227**, 1936

The remarkable absence of symptoms of hypothyroidism in man after total thyroidectomy has called forth several explanatory theories. Among them is the theory that the parathyroid glands undergo metaplasia into thyroid tissue. To test this, the authors performed total thyroidectomy in dogs, removing every trace, including aberrant thyroid tissue. Up to five and one-half months later no change was found in the parathyroids except in 2 cases in which slight compensatory hypertrophy followed accidental removal of half the glands. In 1 case a nodule grossly resembling a parathyroid was found on histologic examination to be a bit of proliferating residual thyroid tissue. Incidentally, the myocardium in these dogs showed no changes after thyroidectomy.

PERRY J MELNICK

EFFECTS OF VITAMIN DEFICIENCY ON THE RETICULOENDOTHELIAL SYSTEM  
U UOTILA and P E SIMOLA Virchows Arch f path Anat **301** 523, 1938

Rats and guinea pigs were maintained on diets deficient in one of the vitamins, A, B or C. One series of young rats received an oversupply of vitamin A. The

animals were vitally stained with lithium carmine before being put to death for histologic study. In A avitaminosis the Kupffer cells of the liver were enlarged and stored the carmine less well than the normal controls. The reticuloendothelial cells of the red pulp of the spleen were hyperplastic, enlarged and contained ingested fragments of erythrocytes. The reticuloendothelial hyperplasia is considered a compensatory reaction to the deficiency or a reaction akin to the keratinization of epithelium. In experiments with deficiency of vitamins B and C and in A hypervitaminosis no definite effects were seen.

O T SCHULTZ

### Pathologic Anatomy

TRUE COR BILOCULARE IN IDENTICAL TWINS F X GIUSTRA and V G TOSTI, *Am Heart J* **17** 249, 1939

Twin babies, who weighed 2,280 and 2,820 Gm, succumbed to identical congenital lesions of their hearts and blood vessels approximately thirty and thirty-nine hours after birth. The cardiac abnormality was true cor biloculare, and the associated anomaly was a persistent truncus. These cases are reported because search of the literature has failed to disclose any similar instance in which identical twins suffered from cor biloculare with persistent truncus.

FROM AUTHORS' SUMMARY

HYPERPARATHYROIDISM DUE TO PARATHYROID ADENOMA WITH DEATH FROM PARATHORMONE [PARATHYROID EXTRACT] INTOXICATION F M HANFS, *Am J M Sc* **197** 85, 1939

An instance of hyperparathyroidism with fatal intoxication due to parathyroid extract is reported. The report fills an existing gap in the natural history of this disease. A characteristic type of parenchymatous renal calcification is illustrated which is believed to be pathognomonic of hyperparathyroidism. A blood calcium level approaching 20 mg per hundred cubic centimeters is indicative of severe and dangerous parathyroid intoxication.

FROM AUTHOR'S SUMMARY

HISTOLOGIC INVESTIGATION INTO THE PYLORIC GLAND ORGAN IN PERNICIOUS ANEMIA E MEULENGRACHT, *Am J M Sc* **197** 201, 1939

It was shown in earlier investigations that the antianemic factor in the stomach (Castle's intrinsic factor) is not found in the pepsin and hydrochloric acid-producing fundus portion but in the pyloric portion and duodenum and that it must be secreted by the pyloric glands and the histologically identical Brunner's glands. The object of the present investigation was a histologic examination of the stomach and duodenum in 8 cases of pernicious anemia, and special regard was given to the pyloric and Brunner glands. Gastritic changes in the fundus portion with atrophy of the glands and disappearance of parietal and chief cells were found in all the 8 cases. But the gastritic changes were less pronounced in the pyloric portion, and the glands seemed relatively well preserved, no histologic changes could be demonstrated in Brunner's glands. How these findings, which at first sight are rather surprising, can be brought into line with the present conception of the genesis of pernicious anemia is discussed.

FROM AUTHOR'S SUMMARY

GASTROSCOPIC OBSERVATIONS IN PERNICIOUS ANEMIA R SCHINDLER and A M SERBY, *Arch Int Med* **63** 334, 1939

Gastroscopic observations on 23 patients with pernicious anemia are presented. Nine of these patients were seen before any treatment had been given, 14 were observed only after adequate treatment, and 3 were examined before and after treatment. All untreated patients presented superficial gastritis, superficial plus

atrophic gastritis or patchy or diffuse atrophy After treatment in 4 patients no marked improvement of the condition of the gastric mucosa was found, in 1 patient there was definite progression of the atrophy, in 7 the mucosa of the antrum was found to be normal, in 1 there was almost complete regeneration, and in 4 all portions of the gastric mucosa became normal

These facts can be explained only by the assumption that in pernicious anemia two separate diseases of the stomach are present Primarily there is dysfunction of the cells which produce the "antianemic" factor Secondly there follows degeneration of the surface epithelium, with superimposed genuine inflammation, which may or may not heal when the deficiency state is eliminated The secondary inflammation usually is combined with a similar disorder of the tongue and of the intestine, with dysfunction of the hemopoietic apparatus and combined degeneration of the spinal cord However, the absence of the "antianemic" factor may sometimes lead to severe but reversible atrophic gastritis, without disease of the blood If this observation is confirmed, the expression antianemic must be replaced by another term

Probably in many cases atrophic gastritis is due to some kind of deficiency state and in some of them to the lack of the "antianemic" factor This disease should be diagnosed by the use of the gastroscope, and in each case in which it is discovered liver therapy should be given tentatively Gastrosopic check of the result of therapy is necessary The frequent incidence of mucosal polyps of the stomach in pernicious anemia is corroborated by the observations of Schindler and Serby

FROM AUTHORS' SUMMARY

"SILVER CELLS" AND "SPIROCHETE-LIKE" FORMATIONS IN MULTIPLE SCLEROSIS AND OTHER DISEASES OF THE CENTRAL NERVOUS SYSTEM G B HASSIN and I B DIAMOND, Arch Neurol & Psychiat **41** 471, 1939

With a modified silver staining method Steiner found what appeared to be spirochetes and silver cells in the brains of patients with multiple sclerosis and dementia paralytica The spirochetes of multiple sclerosis differ, according to Steiner, from those of dementia paralytica They are named by him Spirochaeta myelophthora, for they cause the myelin changes seen in multiple sclerosis and are, in his opinion, its cause S myelophthora is a short-lived organism, it easily breaks up into granules, which are enclosed within lymphocytes or glia cells and are scattered over the visual field, especially near the adventitial spaces of the blood vessels and the ependyma of the cerebral ventricles Steiner's observations were largely substantiated by Hassin and Diamond They, however, found the spirochete-like formations and the silver cells in many other diseases—subacute combined degeneration of the cord, myelomalacia, tumor of the brain, Friedreich's ataxia and many other degenerative conditions The cells containing "silver granules" are neither lymphocytes nor glia cells but are broken up nerve tissue and are present mainly between the axon and the myelin They were found also in and around amyloid bodies, red cells, white cells within the capillaries, adventitial and endothelial cells, the gray substance of the spinal cord and other localities The silver granules, which when arranged in rows resemble spirochete-like structures, are not the cause of multiple sclerosis but its consequence They are probably catabolic products, the earliest manifestation of nerve degeneration, which evidently takes place first between the axon and the myelin G B HASSIN

CONGENITAL CYSTIC DISEASE OF THE LUNG T H SELLORS, Tubercle **20** 49 and 114, 1938

The majority of works on pulmonary disorders contain little reference to cyst formation or cystic disease The term "congenital cystic disease" can best be interpreted as designating an abnormality of lungs in which dilatations or cysts of pulmonary tissue and bronchi are found and to which no infective or parasitic origin can be assigned The designation "congenital" is based largely on the



recognition of many examples of the condition in very young infants, apart from the rarer, but more conclusive, examples that have been found in fetuses. The disruption and derangement of bronchial supporting structures around spaces lined by a bronchial type of epithelium can hardly be confused with any other condition and are highly suggestive of a congenital or developmental origin. This is emphasized to insure distinction between true cystic disease and acquired bronchiectasis. Whereas infection dominates many of the pictures of acquired bronchiectasis, gross dilatations without much infection can occur as the result of bronchial stenosis or of atelectasis. Clinically and roentgenologically a certain amount of confusion is bound to arise. Nomenclature, such as "universal bronchiectasis," "telangiectatic bronchiectasis," "congenital pulmonary lymphangiectasis," "cystic lymphangiomata" and "congenital alveolar adenoma," is given critical consideration. Thirty-two cases are detailed, including 6 of large single cysts, 3 of medium-sized cysts, 16 of multiple small cysts (9 of these without lobar distribution, 3 with patchy distribution and 5 with involvement of a whole lung) and 6 of multiple cysts (diffuse bilateral distribution). An extensive bibliography is appended.

H. J. CORPER

HEMICRANIOSIS B. BROUWER, M. BIELSCHOWSKI and E. HAMMER, *Ann d'anat path* **13** 1, 1936

The article presents a brief review of the literature on a rare condition called hemicraniosis. The clinical and autopsy report of a case is presented. The patient was a 63 year old man. His condition was characterized by internal and external exostoses of the bones of the left side of the skull, both cranium and face, reactive thickening of the meninges of the left side with tumor-like lipomatous nodules, and hypoplasia of the left side of the brain. In view of the strictly unilateral localization, the condition is interpreted by the authors as a congenital defect.

PERRY J. MELNICK

CHANGES IN THE SPLEEN IN THE CIRRHOSIS N. FIESSINGER and R. MESSIMY, *Ann d'anat path* **13** 27, 1936

Fiessinger and Messimy present a discussion of the changes in the spleen in the course of the various types of cirrhosis of the liver, with special reference to fibrosis. The different structures are analyzed (i. e., capsule, trabeculae, arteries, sheathed arteries, veins, sinusoids, lymph follicles and reticulum cells), and emphasis is placed on increase in the thickness, number and complexity of the reticulum fibers. The localization and character of the splenic sclerosis in each of the following conditions are illustrated by the observations in at least one autopsy: Banti's disease, Laennec's cirrhosis, hypertrophic cirrhosis, obstructive cirrhosis, Hanot's cirrhosis, and pigmentary cirrhosis. The influence of both circulatory and irritative (toxic) factors is discussed. Sclerosis of the spleen appears before cirrhosis of the liver in Banti's disease, simultaneously in Hanot's cirrhosis and after the hepatic change in Laennec's cirrhosis.

PERRY J. MELNICK

PATHOLOGIC ANATOMY AND GENESIS OF POTT'S DISEASE R. KAUFMANN, *Ann d'anat path* **13** 81, 1936

On a basis of surgical experiences, postmortem examinations and a study of museum preparations, Kaufmann constructs the following theory of the genesis of Pott's disease. Primarily, the disease is a prevertebral tuberculous lymphadenitis, either mediastinal via the pulmonary route or mesenteric via the intestinal route. By contiguity there is at first superficial caries of the anterior surfaces of the vertebral bodies, this is only later followed by deep caries and the other complex manifestations of Pott's disease. The vertebral caries is therefore not primary. The author has often observed early superficial caries of vertebral bodies adjacent

to tuberculous lymph nodes, without fully developed Pott's disease being present. Tuberculosis of the sternum develops on the same basis, from tuberculous lymph nodes in the anterior mediastinum.

The reason for the development of caries, the author claims, is a mechanical one. Pressure atrophy of the adjacent bone by the inflammatory process is the first step, following which the bone is easily invaded by the tuberculous process. Where no pressure occurs, no caries results, in the lower animals Pott's disease is unknown. For this reason the dorsal decubitus in the treatment of Pott's disease is a mistake, the ventral decubitus, imitating the quadruped's position, yields much better results.

PERRY J. MELNICK

CENTRAL NECROSIS OF THE LIVER G. ROTHE, Frankfurt Ztschr f Path 51 1, 1938

Rothe describes the microscopic picture of central necrosis of the liver and discusses the origin of this lesion. Mallory, who first described it, believed that central necrosis occurred only in severe infection. Other authors showed by experiment that toxins or sensitization, as well as infection, may be responsible. In the author's series of 52 cases, chronic passive hyperemia was present in at least 75 per cent. He points out that in some cases in which myocardial failure or coronary sclerosis is associated with central necrosis there is no toxic, allergic or infective element. Since necrotic tissue is phagocytosed by leukocytes, which are later replaced by connective tissue, central necrosis may be a form of chronic congestion of the liver. The author also believes that there is no morphologic evidence that allergy may be the cause of this lesion.

OTTO SAPHIR

ANATOMY OF PYELONEPHRITIC CONTRACTED KIDNEYS F. LINDER, Frankfurt Ztschr f Path 51 150, 1938

Linder studied the anatomy of the pyelonephritic contracted kidney. The changes occurring in the vessels are emphasized especially. Four stages of changes occurring in the cortex are given, but there is no definite sequence of these stages. Medullary scars may be due to abscesses or to incomplete infarctions, the latter corresponding to destroyed areas in the cortex. Regarding pelvic changes, chronic lesions are mentioned occurring in calices, which appear as foldings of the mucosa with papillary excrescences. Also, so-called cell nests of Brunn are described as evidence of chronic irritation of the pelvis of the kidneys. Linder, in his 3 cases, as well as in others, found hyperplasia of the elastic tissue in the intima, most frequently in medium-sized arteries but also in the larger and the smaller arteries. In the nonaffected portions of the kidneys elastic hyperplasia of the intima in arteries was usually present but less marked than in those of the scars. He explains the appearance of these changes, which occur in the absence of hypertrophy of the heart, as a consequence of so-called "localized hypertension," the result of the disproportion between the amount of blood and the decreased diameter of the renal vessels. The hyperplasia of the elastic fibers in the region of the scars is explained in a similar way. Here the pressure is increased because of the atrophy of the glomeruli and the numerical decrease in arteries in the corresponding scars. The most striking change in the vessels of the regions of the scars, however, was lipoidosis. The arterioles in the contracted foci were the most affected. For the most part these changes were present at points of division, while the parts just proximal to the glomeruli were not involved. Linder stresses that in hypertension the arterioles were narrowed particularly at points of division. If the vessel contracts at this point, the flow of blood, which is increased because of the closure of neighboring arterioles, may stop. Because these narrower points present sites of lessened resistance they undergo fatty degeneration. The author believes that for these reasons the arteriosclerosis is not the cause but the consequence of the hypertension.

OTTO SAPHIR

ROLE OF VASCULAR HAMARTIA IN THE GENESIS OF INTERNAL HEMORRHAGIC PACHYMENINGITIS P HEILMANN, Virchows Arch f path Anat **301** 547, 1938

Systematic microscopic examination of the dura revealed small telangiectatic angiomatous capillary maldevelopments immediately beneath the inner surface in 2 to 3 per cent of the cases Heilmann thinks hemorrhage from such vessels, resulting from toxic degenerative changes in the endothelium or from slight trauma, may be a cause of chronic hemorrhagic internal pachymeningitis

O T SCHULTZ

### Microbiology and Parasitology

MATRIX OF INCLUSION BODY OF TRACHOMA P THYGESON, Am J Path **14** 455, 1938

The inclusion body found in the epithelial cell in trachoma contains a ground substance or matrix which is composed predominantly of glycogen This matrix is apparently absent or present only in low concentration in young inclusions and uniformly present in mature ones Its formation seems to be associated with the change from the large initial body form of the virus to the small elementary body form In view of the ease with which these inclusion bodies may be recognized with low magnification, the iodine stain should prove distinctly valuable as a laboratory test in the diagnosis of trachoma

FROM AUTHOR'S SUMMARY

INFECTIVITY OF THE PRIMARY COMPLEX OF TUBERCULOSIS W H FELDMAN and A H BAGGENSTOSS, Am J Path **14** 473, 1938

The lesions of the primary complex of tuberculosis when definitely encapsulated and sclerotic or caseous or caseocalcareous seldom contained viable or virulent organisms of *Mycobacterium tuberculosis* Neither the presence nor the absence of viable or virulent organisms of *Mycobacterium tuberculosis* in the lesions of the primary complex of tuberculosis can be established by morphologic appearances alone The data suggest that in adults endogenous reinfection is unlikely to occur from lesions of the primary complex Silica in varying amounts is found fairly constantly in lesions of the primary complex of tuberculosis In the absence of demonstrable viable tubercle bacilli in the lesions it is suggested that histologic signs of activity are possibly due to silica

FROM AUTHORS' SUMMARY

ULTRACENTRIFUGATION OF VACCINE VIRUS J E SMADEL, E G PICKELS and T SHEDLOVSKY, J Exper Med **69** 607, 1938

Ultracentrifugal studies of the CL dermal strain of vaccine virus warrant the following conclusions When suspended in increasing strengths of sucrose, glycerol or urea solutions, elementary bodies of vaccinia show variations in sedimentation rate which indicate changes in the density or size of the particles For a given change in the density of the medium these changes are smallest with sucrose and most marked with urea The normal rate of sedimentation of Paschen bodies may be restored by resuspending them in dilute buffer solution The density of elementary bodies of vaccinia suspended in dilute buffer solutions is estimated to be 1.16 Gm per cubic centimeter Higher values for density are found if the particles are suspended in solutions containing sucrose, glycerol or urea In 53 per cent sucrose, for example, the density is 1.25 Gm per cubic centimeters Paschen bodies appear to be quite permeable to water and urea, less so to glycerol and only slightly, if at all, to sucrose The increased density of the elementary bodies of vaccinia in sucrose solutions may be accounted for by an osmotic extraction of water from the particles On this basis the water which can be thus extracted corresponds to at least a third of the original volume of the particles

FROM AUTHORS' SUMMARY

A PECULIAR TYPE OF HEMOLYSIS PRODUCED BY STAPHYLOCOCCUS AUREUS (L'HEMOPHAGIE STAPHYLOCOCCIQUE DE MULLER) T PACKALÉN, *Acta path et microbiol Scandinav* **15** 9, 1938

Packalen studied a little known phenomenon, first described by Muller in 1927, which concerns slowly developing discrete secondary hemolytic zones produced by staphylococci on blood agar plates. He has verified the findings of Muller concerning the dependence of this hemolysis on a chemolabile and thermolabile filtrable substance in the blood of certain animal species. The demonstration of this hemolysis occurs only when erythrocytes of certain species are used, the author making the interesting observation that corpuscles sensitive to secondary hemolysis are high in potassium and low in sodium content, while the reverse proportions characterize resistant corpuscles. The supposition of this investigator is that these areas of hemolysis are "the visible manifestation of invisible virus colonies deriving from staphylococcal mother colonies. The animate or inanimate nature of this virus is left open to discussion." A recent communication from the author also reveals that this phenomenon can be elicited not as hemolytic zones but as clear zones in opalescent heated serum plates without the addition of erythrocytes or hemoglobin. The reviewer, in working on the same problem, has been inclined to believe, as does Muller, that the primary element is to be found in the serum, since a quantitative relationship depends on this substance, the staphylococci activating this element, probably depending on some peculiar metabolic activity, as evidenced in variations of mannite fermentation by active and nonactive strains.

G BERNICE RHODES

### Immunology

TUBERCULOUS ALLERGY WITHOUT INFECTION F R SABIN and A L JOYNER, *J Exper Med* **68** 659, 1938

Guinea pigs can be rendered hypersensitive to tuberculoprotein by repeated intradermal injection of small amounts of active tuberculoprotein. Tuberculo-phosphatide added to the protein speeds up the sensitization and enhances it so that the reactive tissues become indurated and necrotic, closely simulating tissues involved in the disease. Active tuberculoproteins induce new formation of monocytes and of some epithelioid cells. The addition of phosphatide to the protein brings about massive formation of epithelioid cells. With the increase in the cellular reaction to the injection of the mixed phosphatide and protein may be correlated the increase in the speed and intensity of the sensitization. The intradermal route is the best for such sensitization, probably because it provides the greatest dose of the sensitizing agent per cell. The degree of sensitization artificially obtainable by the synergistic action of tuberculo-phosphatide and tuberculoprotein is quite comparable to the degree of sensitization occurring naturally in tuberculous animals, moreover, this degree of sensitization may be induced with amounts of the materials from the bacilli which could conceivably be present in the tissues of an infected host.

FROM AUTHORS' SUMMARY

ANTIBODY PURIFICATION M HEIDELBERGER, P GRABAR and H P TREFFERS, *J Exper Med* **68** 913, 1938

Tested quantitatively, antibody recovered by dissociation of specific precipitates from antipneumococcus serum reacts with homologous polysaccharide almost as does the antibody in the original serum. The dissociation methods employed appear to yield a portion of all the anticarbohydrates of differing reactivities in the serum, rather than a fraction of low or high reactivity. The reversible inactivation of purified pneumococcus anticarbohydrate by formaldehyde is confirmed, and the failure of data on the formaldehyde-antibody reaction to permit a choice between alternative theories of specific precipitation is shown.

FROM AUTHORS' SUMMARY

PRECIPITINOGEN IN SERUM PRIOR TO RHEUMATISM A F COBURN and R H PAULI, *J Exper Med* **69** 143, 1939

Between serum taken just before and serum taken shortly after the onset of acute rheumatism a precipitin reaction occurs. It recurs with repeated rheumatic cycles. Certain properties of the precipitinogen and precipitin are described.

FROM AUTHORS' SUMMARY

EFFECT OF SPLENECTOMY AND BLOCKADE ON THE PROTECTIVE TITER OF ANTISERUM AGAINST *TRYPANOSOMA EQUIPERDUM* L R KUHN, *J Infect Dis* **63** 217, 1938

The protective titer of sheep anti-*Trypanosoma equiperdum* serum when injected intraperitoneally into mice is appreciably reduced if the mice are splenectomized and blockaded with india ink or normal serum and inoculated subcutaneously with 2,500 to 3,000 trypanosomes, but is not reduced if the mice are only splenectomized or if they are splenectomized and blockaded with specific (1 e, sheep anti-T *equiperdum*) serum. Furthermore, it is only slightly reduced in mice that are splenectomized and blockaded with india ink and given a subcutaneous injection of 1,200 to 1,600 trypanosomes. Splenectomy plus blockade appears to have no effect on the resistance of mice to trypanosomes specifically sensitized in vitro. The effect of splenectomy and blockade on the protective titer of trypanocidal serum cannot, therefore, be due to inhibition of phagocytosis, decrease of complement or reduction of opsonin, but it may be due to interference with the concentration of passively acquired antibodies in macrophage tissues.

FROM AUTHOR'S SUMMARY

PNEUMOCOCCUS TYPE XIV AND HUMAN RED CELLS M FINLAND and E CURNEN, *Science* **87** 17, 1938

This study was undertaken to explain the unusual reactions, occasional fatalities and, in 1 case, hemoglobinuria following the use of antipneumococcus XIV horse serum. Finland and Curnen found that horse antiserum for *Pneumococcus* type XIV had the capacity of agglutinating human bloods of all four groups at titers ranging from 1:80 to 1:260. On the other hand, of 41 antisera for pneumococcus types other than XIV, only 2 agglutinated human cells at dilutions of 1:20 or higher. Rabbit antipneumococcus sera did not exhibit this phenomenon.

A S WIENER

SURFACE FILMS OF ANTIBODIES AND ANTIGENS J F DANIELLI, M DANIELLI and J R MARRACK, *Brit J Exper Path* **19** 393, 1938

Surface films of pneumococcus (type II) antibody spread at air-water and oil-water interfaces show no sign of ability to combine with the specific polysaccharide. Surface films of horse serum globulin show no sign of ability to combine with a highly active rabbit antiserum.

FROM AUTHORS' SUMMARY

REACTIONS BETWEEN DIPHTHERIA TOXIN AND ANTITOXIN C G POPE and M HEALEY, *Brit J Exper Path* **19** 396, 1938

A photoelectric method has been used to study various phases of the Ramon flocculation reaction between diphtheria toxin (or toxoid) and antitoxin. The following observations were made. The production of opacity is more rapid in a mixture containing a dose of antitoxin equivalent to that of toxin than in one in which the dose of antitoxin is either more or less than that of toxin. The rate is affected more by a decrease than by an increase in the amount of antitoxin, probably because mixtures containing two equivalents eventually develop a greater opacity than the mixture containing one equivalent. The rate is increased if the

antitoxin is added in two fractions. A preformed equivalent mixture gave high reactivity with more antitoxin, and under certain conditions a second stage may be observed, which is the resolution of preformed aggregates by excess antitoxin. Mixtures of toxin and antitoxin in varying proportions at equilibrium gave opacity curves similar in shape to the curves for the nitrogen content of flocules of varying composition, the maxima occurring at approximately the same place. This method of investigation is being used in further studies of precipitation reactions.

FROM AUTHORS' SUMMARY

SENSITIVITY OF RHEUMATIC SUBJECTS TO STREPTOCOCCAL PRODUCTS C. A. GLEN,  
J. Path. & Bact. **47** 337, 1938

Of 32 patients with acute rheumatism, 27, 14 and 13 showed cutaneous sensitivity to the endotoxin of autogenous hemolytic, viridans and indifferent streptococci, respectively. Seventy-five per cent of a series of 105 patients with acute and subacute rheumatism gave positive skin reactions with a stock preparation of the endotoxin of the hemolytic streptococcus as compared with 24 per cent of 105 nonrheumatic controls. In a limited number of persons whose rheumatic disease had become quiescent, local and general manifestations of the rheumatic syndrome have been induced by subcutaneous injection of the endotoxin of the hemolytic streptococcus. The evidence supports the view that an infection with the streptococcus is an important factor in the production of the rheumatic state.

FROM AUTHOR'S SUMMARY

HEREDITY OF THE SUBGROUPS  $A_1$  AND  $A_2$  P. DAHR and W. BUSSMANN, *Ztschr. f. Rassenphysiol.* **10** 49, 1938

Dahr and Bussmann report the results of studies on the heredity of the subgroups of group A and group AB in 106 families with 448 children. In this series there was a family in which the parents belonged to group A and group O, respectively, and the child to group AB, this was attributed to illegitimacy. In addition there were 2 exceptions to the theory of Thomsen, Friedenreich and Worsaae as to the heredity of the subgroups: (1) a child belonging to subgroup  $A_1$  who had parents of subgroup  $A_2$  and group O, respectively, (2) an  $A_2$  mother who had an  $A_1B$  child. The last-named exception could not be attributed to illegitimacy, since under the aforementioned theory  $A_2$  parents cannot possibly have  $A_1B$  children, and the exception in question involved the mother, not the father. The relatively large number of exceptions encountered in this study and also in those previously published on the heredity of the subgroups indicates the need for further studies before the subgroups can be safely applied in medicolegal cases.

A. S. WIENER

## Tumors

SYNOVIAL SARCOMA IN SEROUS BURSAE AND TENDON SHEATHS L. BERGER,  
*Am. J. Cancer* **34** 501, 1938

Berger reports 5 cases of synovial sarcoma in which the cytologic observations were distinctive. The origin was traced definitely to the synovial membranes of serous bursae in 3 cases and probably in the fourth case, while in the fifth case the growth originated in a tendon sheath. The histologic features could be arranged in several groups: (a) A syncytium-like structure identical with that of some reticulosarcomas of bone marrow or lymph nodes. (b) Areas resembling an irregular type of fibrosarcoma or rather of histiocytoma. (c) Cavities lined with cuboidal or columnar pseudoepithelial cells, which were in all other details identical with the cells lying beneath them. The cavities contained mucin mixed with cellular and nuclear debris, occasionally with calcium incrustations. The mucin was apparently not produced by the cells but arose in the intercellular substance. (d) Large foamy cells with lipophagic and siderophagic properties.

Not all these features were present in every case. All cases presented malignant features. The fifth tumor contained in addition multinucleated giant cells of the type seen in so-called giant cell tumors of tendons. This case is, according to Berger, the first instance of malignant xanthomatous giant cell tumor on record. The histogenetic diagnosis was reticuloendotheliodhistiocytosarcoma.

Only 23 cases, including the 5 reported here, with specific synovial features are to be found recorded in the literature. In 11 of the cases the tumor originated in a joint, in 9, in a bursa, and in 3 in a tendon sheath. In 16 the process was definitely malignant. Berger suggests a classification into (1) benign synovial histiocytoma (with histiocytic features predominating and neoplastic nature undetermined), (2) synovialoma (neoplastic, with predominantly endothelial, or synoviothelial, or mucous properties) and (3) malignant synoviosarcoma (with histiocytic [xanthomatous, with or without giant cells], endothelial, muciparous or different features), the latter in some instances being indistinguishable from common fibrosarcoma.

I DAVIDSOHN

#### MESONEPHROMA OVARII W. SCHILLER, *Am J Cancer* **35** 1, 1939

Schiller reports a new type of ovarian tumor, observed in 19 patients, which is characterized by structures resembling the renal glomerulus as it is seen in the wolffian body or mesonephros. In addition to the morphologic similarity, embryologic considerations favor derivation from the mesonephros. The basic structure is that of a cystic cavity lined with flat cells having bulging nuclei and relatively scanty cytoplasm. The latter is strikingly thinned out on the sides of the nuclei, resulting in a wavy border. Into these cavities one or two papillary structures protrude, carrying capillaries and lined by cells similar to those on the inner wall of the cyst. One case was noted in which forms resembling renal tubules were found. Embryologic and functional considerations explain the relative infrequency of tubular forms in mesonephroma. In 1 instance the tumor was associated with a dermoid cyst and in 1 instance with an embryoma. Experience to date suggests that the growth is of a malignant nature. The age of the patients varied from 8 months to 69 years. Schiller believes that mesonephroma is not rare.

I DAVIDSOHN

#### MAMMARY GLAND IN HIGH AND LOW TUMOR STRAIN E. FEKETE, *Am J Path* **14** 557, 1938

A comparative morphologic study of the mammary glands of the Little-Murray dilute brown high tumor strain and the C57 black low tumor strain shows that the cells of the mammary tissues of the former do not respond so uniformly to the endocrinal influences that regulate the progressive, functioning and regressive changes of the gland as do those of the low tumor strain. In the high tumor strain groups of cells may persist in cell division while all the others are already functioning, or they fail to regress, sometimes continuing to function while all the others have undergone regression. Persistent mitotic activity of groups of cells leads to early malignant changes.

FROM AUTHOR'S SUMMARY

#### LYMPH NODE METASTASIS OF SARCOMA S. WARREN and R. W. MEYER, *Am J Path* **14** 605, 1938

Metastasis in lymph nodes occurs in 5 to 10 per cent of all hospital cases of sarcoma (exclusive of melanomas, lymphosarcoma and clinically benign leiomyosarcoma of the uterus). Seventeen cases of microscopically demonstrated metastasis in lymph nodes are presented. One patient is now living six years after removal of the involved nodes. Dissection of the involved lymph nodes with radical removal of the sarcoma may improve the prognosis in from 5 to 10 per cent of cases and may be valuable even after lymphatic metastasis is clinically evident.

FROM AUTHORS' SUMMARY

CARCINOMA OF THE LUNG S KOLETSKY, Arch Int Med **62** 636, 1938

The histologic classification of primary carcinoma of the lung may be correlated with essential differences in growth, dissemination and prognosis

The small cell carcinoma is highly malignant and offers a poor prognosis. It is usually primary at the hilus of the lung, it readily invades the postero-superior mediastinum and metastasizes extensively, with an especial tendency toward widespread extension through the lymphatic system. The tumor occurs in younger persons. There is a short, rapid course without notable remission.

The squamous cell carcinoma is slow growing, locally invasive and relatively nonmetastasizing. It is accompanied by infection, necrosis and cavity formation. Involvement of lymph nodes other than the regional and tracheobronchial nodes is infrequent. This type of carcinoma, particularly when it involves the hypo-arterial bronchus of a lower lobe of a lung, offers the most favorable prognosis for complete resection.

Adenocarcinoma is less favorable to surgical intervention, since, while locally invasive, it more frequently and more extensively involves the lymph nodes and metastasizes vigorously by way of the blood stream.

FROM AUTHOR'S SUMMARY

ESTROGENIC HORMONES AND CARCINOGENESIS L A EMGE, Surg, Gynec & Obst **68** 472, 1939

In a series of experiments on white rats of different ages in which aqueous theelin and theelin in oil were administered in varying doses and over varying periods of time, no malignant changes were produced in the mammary glands, genital tract or a transplanted mammary adenofibroma. Since this strain of rats is entirely free from spontaneous cancer, it is assumed that a hereditary immunity protects the breast and genital tissue against excessive and uncontrolled proliferation, regardless of massive doses of estrogen. The proliferative changes observed are quantitative and self limited. They probably do not occur spontaneously even under high physiologic loads of estrogen, as observed in experiments on pregnancy. A maximal response to estrogenic stimulation is terminated by a process of cell exhaustion and cell destruction, probably dependent on changes in the hypophysis due to superphysiologic stimulation by estrogen. Evidence is accumulating to prove that the action of the estrogenic hormones is controlled by definite biologic patterns and that their cancer-provoking faculty in small laboratory animals is strictly limited by hereditary tendencies. There also is evidence that the effect of estrogen on mammary and genital epithelium is essentially quantitative. The carcinogenic effect of estrogen is believed to be limited. Because this hormone favors spontaneous mammary cancer in mice highly susceptible to this malignant development is not proof that it operates in like manner in other species of mammals.

FROM AUTHOR'S SUMMARY (WARREN C HUNTER)

BENZPYRENE AND TRANSPLANTED CARCINOMA P R PEACOCK and S BECK, Brit J Exper Path **19** 434, 1938

There is evidence that intravenous injections of 3,4-benzpyrene colloid can inhibit transplanted mouse squamous carcinoma 2146, success depending more on the frequency of the injections than on the amount injected, in 6 of 13 mice that received eight or more injections of colloid, four tumors regressed completely and two partially. The toxicity of intravenously injected benzpyrene colloid differs in the various species of animals tested, being low in mice, rabbits and fowls (and in 1 human being) and high in rats and goats. Intravenously injected benzpyrene colloid is not harmless, three pulmonary adenomas observed in a mouse three hundred and fifty-four days after the first injection were probably attributable to the treatment. Deposits of benzpyrene can be demonstrated in the lungs of mice shortly after intravenous injection of benzpyrene colloid.

FROM AUTHORS' SUMMARY



MENINGIOMA J R M INNES, W F HARVEY and E K DAWSON, Edinburgh  
M J **45** 855, 1938

Meningioma arises from stem cells of the pachymeninx or leptomeninx, i. e., from membranes of mesodermic origin. These stem cells are of a peculiar type, individual to the meninges. Meningioma is not, therefore, fibroblastoma, endothelioma, hemangioblastoma or glioma, but is derived from a type cell of meningeal, mesodermic origin, which the authors name specifically meningoblast or meningocyte.

FROM AUTHORS' SUMMARY

TUMORS IN MICE A HADDOW, J Path & Bact **47** 553, 1938

The following conclusions are based on a study of 336 spontaneous tumors in mice, 331 of which were mammary tumors. The occurrence of multiple primary neoplasms approached a chance distribution, although the approximation was statistically inadequate to justify the assumption of actual random incidence. In the great majority of cases the linear measurements of tumor size increased linearly with time, although a small proportion showed exponential increase. No significant association could be established between the rate of growth and incidence of metastases, although such an association was present in a series studied by Ashburn. A significant correlation was found between size of tumor and incidence of metastasis. The duration of the primary tumor was found to be a highly important single factor in determining the occurrence of metastasis. No significance could be attributed to an apparent increase in the frequency of metastasis in mice with multiple primary tumors. Tumors situated in the four breasts in the caudal half of the body were observed to possess a mean growth rate significantly higher than the mean for similar tumors in the six mammary glands cephalic in position. No relation was found to exist between location of tumor and production of metastasis. When tumors of extreme types were studied, it appeared justifiable to conclude that those of differentiated adenomatous structure more often possessed low rates of growth than the dedifferentiated and anaplastic histologic types. In pregnant animals bearing spontaneous mammary carcinoma no evidence was found to suggest that gestation influenced the rate of tumor growth, but parturition and the onset of lactation were uncommonly followed by temporary retardation. Slow growth was frequently observed in the earliest stages of tumor development, such examples attaining their maximal rate only after a variable and often considerable interval. Mention is made of the possible etiologic significance of this phenomenon. Apart from the effect described as consequent on parturition and lactation retardation during the later stages of growth was mainly due to incidental factors, such as bacterial infection of the tumor substance.

FROM AUTHOR'S SUMMARY

INHIBITION OF TUMORS BY CARCINOGENS A HADDOW, J Path & Bact **47** 567 and 581, 1938

Parenteral administration of 1,2,5,6-dibenzanthracene in mice bearing spontaneous neoplasms (mainly carcinomas of the mammary gland) resulted in most cases in a prolonged inhibition of tumor growth. The degree of individual response varied from slight to marked, and in a few animals there was active regression, partial or complete. The same result, with a corresponding degree of variation, was produced by the carcinogenic substances 1,2,5,6-dibenzacridine and styryl 430. The noncarcinogenic compounds pyrene and 1,2,3,4-dibenzanthracene either did not provoke a response or occasioned only transient interference with tumor growth, which was followed by recovery to the previous rate. Examples were met with in which the noncarcinogenic substances acenaphthanthracene and 1,2,5,6-dibenzphenazine produced a retardation of growth not different from that produced by carcinogenic compounds. Administration of large doses of estrone benzoate produced in some cases no inhibition and in others moderate retardation of tumor growth with a tendency toward rapid recovery.

The data indicate that primary chemically induced sarcomas as a class tend to be considerably less susceptible to the inhibitory action of carcinogenic substances.

than spontaneous or transplanted tumors This relative resistance is not, however, specific, since tumors induced by a given carcinogenic compound were not significantly more resistant to the inhibitory action of the same compound than to that of other carcinogenic substances

FROM AUTHOR'S SUMMARIES

TUMORS AND LYMPHOGRANULOMA VIRUS R SCHOEN, Ann Inst Pasteur **60** 499, 1938

In mice in which sarcoma material is engrafted subcutaneously or intrapeitoneally and which simultaneously are inoculated intracranially with lymphogranuloma virus the virus becomes fixed to the developing sarcomatous neoplasms The proliferating sarcomatous elements provide favorable conditions for abundant development of the virus in the neoplastic tissue The numerous serial passages of lymphogranuloma virus by grafting tumors thus contaminated in vivo, the high virulence of the virus and the persistence of this virulence after repeated washing of the neoplastic tissue furnish evidence that there is a true culture of virus in vivo within the sarcomatous cells The multiplication of the lymphogranuloma virus within the tumor tissue does not modify the proliferation of the neoplastic cells

FROM AUTHOR'S CONCLUSIONS

SARCOMATOUS EPULIS L GERY, Bull Assoc franç p l'étude du cancer **27** 124, 1938

Gery found reports of one sarcomatous epulis that was established to be such and one that was possibly in the literature He adds a report of 4 cases observed by him

1 A 71 year old man had an involvement of the upper gum No other clinical data were available Histologically the lesion showed small cells with large nuclei, which were round or slightly elongated, frequent mitoses, and no giant cells The diagnosis was small cell fibrosarcoma (*sarcome histiocyttaire à petites cellules*) A possible origin from the alveolar process was considered but excluded

2 A man aged 36 had a diffuse swelling of the left lower gum with involvement of the cervical lymph nodes on the same side Biopsy showed a densely cellular growth with extreme cellular and nuclear irregularities and many multinucleated giant cells with similar nuclear irregularities The giant cells did not resemble those of epulis There were occasional foam cells A biopsy specimen from a metastasis in a lymph node showed an identical histologic picture The diagnosis was polymorphous fibrosarcoma (*sarcome histiocyttaire polymorphe*) The patient died five months after the onset, with multiple cutaneous metastases and with invasion of the tongue No necropsy was made

3 A very cellular growth was found in the lower gum of a man of unknown age Side by side with giant cells of the epulis type were, in large numbers, mononucleated and multinucleated true tumor giant cells with pronounced morphologic, tinctorial and nuclear irregularities, with abundant atypical mitoses and with foci of new-formed osteoid tissue There were no clinical data The diagnosis was giant cell polymorphous fibrosarcoma

4 A gingival tumor was found invading the maxilla of a 58 year old man It was a very cellular growth with extreme variations as to size, shape and staining of the cells and particularly of the nuclei There were very few mitotic figures Many vascular spaces were lined by the tumor cells There were evidences of destruction of bone and many fibrous areas The diagnosis was giant cell fibroblastic sarcoendothelioma (*sarco-endothelioma histiocyttaire, gigantocellulaire et fibroblastique*)

I DAVIDSOHN

HISTOLOGIC DIAGNOSIS OF EMBRYONAL LIPOMA G GRICOUROFF, Bull Assoc franç p l'étude du cancer **27** 251, 1938

An encapsulated tumor with a tendency to recur and occasionally even to metastasize which originates wherever adipose tissue is present but is seen most

frequently in the thigh and in the retroperitoneal space can be easily mistaken for myxoma unless frozen sections are stained for fat, for it resembles myxoma closely in the gross and even microscopically. Droplets varying in size from very small ones to such large ones as to fill the entire cell are found, they are more numerous away from the blood vessels. Mucicarmin staining shows the characteristic color reaction in the interstitial tissue but never in the protoplasmic droplets. That finding is, according to Masson, pathognomonic for true myxoma. The fat droplets in embryonal lipoma or, as it is sometimes called, myxomatous lipoma are not due to degenerative changes in the nuclei. They are a product of cellular secretion and are pathognomonic for the lipoma. It may be necessary to consider 1 A myxoma that invades fat tissue. In that case there will be compact lobules of adult adipose tissue, but there will be no fat droplets in the young tumor cells. 2 An undifferentiated sarcoma with fatty degeneration. In that case other signs of degeneration and of necrosis will be found. 3 Xanthogranuloma. The spongiocytes (foam cells) characteristic of the latter will help to make the correct diagnosis. Some authors claim that embryonal lipoma is essentially benign and that the recurrences and metastases are evidences of a multicentric, systemic nature of growth.

I DAVIDSOHN

### Medicolegal Pathology

QUESTION OF THE RIGHT OF A CORONER TO AUTHORIZE AN AUTOPSY WITHOUT HOLDING AN INQUEST. *Patrick v Employers Mutual Liability Insurance Company (Mo)*, 118 S W (2d) 116

Patrick, a fireman employed by the City of Macon, Mo, collapsed and died while fighting a fire. The local physician of the defendant insurance company which insured the city against possible liability under the Missouri workmen's compensation act notified the coroner that the city had insurance on Patrick and that "they ask for an autopsy." Later in the day another physician employed by the insurer performed the autopsy. Patrick's widow sued the insurance company, alleging that it had caused the autopsy to be performed without her consent or knowledge. From a judgment for the widow the defendant appealed to the Kansas City court of appeals, Missouri.

The court of appeals found no merit in a contention that the widow's remedy was exclusively under the workmen's compensation act. This suit, said the court, is based on the theory that the widow has a quasi-property right in the remains of her husband. The wrong of which she complains occurred after the death of the husband, was in no way connected with the cause of his death and could not therefore be cognizable under the compensation act.

The court disagreed with a contention made by the insurance company that the autopsy was legally performed. In Missouri, the court pointed out, a coroner has no authority to order an autopsy except in connection with an inquest to be held before a coroner's jury. No inquest was held in this case, and it was evident that the coroner had no intention to hold an inquest, for he testified that the autopsy was performed merely that he might have information on which to make out a death certificate.

The insurance company next insisted that if the coroner believed an autopsy was necessary he acted in his judicial capacity in having one performed, and the defendant insurance company was therefore protected. But, answered the court, a coroner acts judicially only with respect to determining whether an inquest shall be held and not in the "calling or holding of an autopsy." He has no authority to order an autopsy except in connection with an inquest. In the opinion of the court, the jury could conclude from the evidence that even though the coroner sought to justify the autopsy on the theory that it was necessary for him to sign a death certificate, he abused his office in authorizing the autopsy for the purpose of assisting the defendant insurance company to obtain evidence to be used to defeat any claim for compensation to be made by the widow.

For the reasons stated, the judgment in favor of the widow was affirmed.

FROM J A M A

# Society Transactions

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## BUFFALO PATHOLOGICAL SOCIETY

ERNEST WITEBSKY, *President*

*April 15, 1939*

SAMUEL SANES, *Secretary*

### TREATMENT OF ACUTE INFECTIOUS MONONUCLEOSIS WITH SULFANILAMIDE STUART L. VAUGHAN and HOWARD OSGOOD

Five patients with acute infectious mononucleosis were treated with the full dose of sulfanilamide. All of these patients had the sporadic type occurring in young adults. In each the symptoms were moderately severe, with fever, prostration and lymph node enlargement. The blood picture was typical. The titer of heterophil agglutination was 1:640 or higher. In 4 patients pharyngeal or oral ulcers were present. In 3 patients the spleen was demonstrably enlarged.

In 2 cases in which administration of the drug began early in the second week a prompt remission of clinical symptoms followed, with a crisis-like fall in the temperature and rapid healing of the ulcers. In a case without ulceration in which treatment was started on the first day of illness the clinical improvement was rapid, but the fever persisted for eleven days. Atypical hemolytic anemia developed, with the hemoglobin falling to 62 per cent, but the hemoglobin rose again steadily despite continued administration of the drug. In a case in which the treatment was started on the seventh day the ulcers healed rapidly and there was clinical improvement until a severe drug eruption developed and interfered with subsequent evaluation of the symptoms. In a case with severe ulceration, in which treatment was started on the thirtieth day of illness, no improvement was noted during the succeeding nine days. In all of the cases the blood and lymph nodes returned to normal no more rapidly than would be expected in cases in which no treatment was given.

### OBSERVATIONS ON CHILDREN WITH INFLUENZAL MENINGITIS WHO WERE TREATED WITH SPECIFIC SERUM, SULFANILAMIDE AND SULFAPYRIDINE ERWIN NETER

During the last two years at the Children's Hospital 12 patients with influenzal meningitis were observed, and some were treated with specific serum, sulfanilamide and sulfapyridine (2-[paraaminobenzenesulfonamido]-pyridine), respectively. The age of the patients varied from 3 months to 4 years, of the 12 patients, 9 were below 2 years of age. During the same period no patient with influenzal meningitis was admitted to the Buffalo General Hospital. This confirms the observations of others that influenzal meningitis most frequently occurs in early childhood.

The spinal fluid of the 12 patients prior to treatment was found to be cloudy. The cell count varied from 304 cells per cubic millimeter in one case to 17,000 cells per cubic millimeter in another. Bacilli were seen in the sediment of the spinal fluid in all cases, but their number varied markedly with the particular specimen. Bacilli were most numerous in spinal fluids containing relatively few cells. Specific soluble substance was demonstrable in all spinal fluids. Mention may be made of the occurrence of a zone phenomenon, i. e., undiluted anti-Haemophilus influenzae serum may fail to give specific precipitation, while serum diluted 1:10 may cause definite precipitation, with the supernatant of the spinal fluid.

Of these 12 patients, 9 seem to have had "primary" influenzal meningitis. One of the other 3 patients three weeks prior to the appearance of meningeal signs suffered from H. influenzae bacteremia, with metastatic purulent lesions in several

joints In a second patient tracheobronchitis which necessitated tracheotomy preceded the meningitis, and *H influenzae* was cultured from the tracheal exudate The third patient had tracheitis caused by a hemolytic streptococcus prior to the onset of the influenzal meningitis

The duration of the disease in the 12 patients varied from three days to five weeks All the patients died in spite of treatment with specific serum, sulfanilamide and sulfapyridine, respectively Attention may be called to the acute fulminating form of influenzal meningitis, these patients died within three to five days after the appearance of the first signs of the disease

The anti-*H influenzae* serum used in the treatment of these patients was obtained from the Massachusetts Commonwealth, Boston, through Dr L Fothergill It was administered intrathecally, together with normal human serum as complement, and in some of the patients also intravenously Sulfanilamide was given by mouth and also intrathecally as a 0.8 per cent solution in physiologic solution of sodium chloride Sulfapyridine was given by mouth In some of the cases the administration of the specific serum resulted not only in clinical improvement but also in parallel changes in the spinal fluid First, the number of bacilli present in the spinal fluid decreased, and finally the culture became sterile At the same time, the sugar concentration in the spinal fluid increased, and the cell count decreased However, in all cases reported in this series, in spite of continued treatment, bacilli invariably reappeared, followed later by a decrease in the concentration of sugar in the spinal fluid With the reappearance of micro-organisms the cell count may or may not increase In no case was a second sterilization of the spinal fluid achieved

One of the patients was treated exclusively with sulfanilamide, given by mouth and by intrathecal injection, and later with sulfapyridine At no time did cultures of the spinal fluid show that it had become sterile The specimens of spinal fluid of this patient were used in an attempt to test the action of sulfanilamide and sulfapyridine on the viability of *H influenzae* present in the spinal fluid To this end, spinal fluid containing various concentrations of sulfanilamide and sulfapyridine, respectively, were incubated at 37 C At various intervals subcultures were made on chocolate agar plates The latter were incubated at 37 C in a jar containing about 10 per cent carbon dioxide These preliminary experiments revealed that *H influenzae* remained viable in spinal fluid containing sulfanilamide in concentrations of from 5 to 15 mg per hundred cubic centimeters even when it had been exposed to the action of sulfanilamide for twenty-four hours In spinal fluid containing sulfapyridine, however, the organism lost its viability, provided it was exposed to the action of the drug for twenty-four hours Further experiments are necessary in order to assay the action of these drugs on *H influenzae* present in spinal fluid and to elucidate the matter of whether or not strains of *H influenzae* differ in their susceptibility to the action of the drugs

NOTE—Since this abstract was submitted for publication 2 additional cases of influenzal meningitis have been observed One patient died, the other ultimately recovered The latter, a boy 18 months of age, was treated with a total of 213 cc of anti-*H influenzae* serum, given intravenously and intrathecally, and a total of 205 Gm of sulfapyridine, administered over a period of twenty-seven days On the third day of treatment the spinal fluid became sterile On the seventh day, however, *H influenzae* reappeared in the spinal fluid Under continued treatment with specific serum, sulfapyridine and sulfanilamide (the latter being given intrathecally) the spinal fluid finally became sterile, and the patient made a complete recovery

#### FERMENTABLE SUGARS (DEXTROSE AND FRUCTOSE) IN PERICARDIAL FLUID ROGER S HUBBARD and EMERSON HOLLEY

One of us has shown that, in addition to dextrose, there are large amounts of fructose in spinal fluid and that in contradistinction to the relationship between dextrose in the blood and dextrose in the spinal fluid the concentration of fructose

is much greater in the spinal fluid than in the blood. It has not as yet been found possible to explain adequately the presence of fructose in spinal fluid. Various factors may have resulted in the difference noted between blood and spinal fluid. For example, fructose may be formed from dextrose during the passage of the latter sugar through the capillary wall. Another possible explanation is that a tendency toward a condition of equilibrium between dextrose and fructose exists in body fluids which leads to the production of measurable amounts of the latter sugar in the absence of red blood cells in high concentration. A third possibility is that the formation of fructose from dextrose results directly from enzymatic processes or from cellular metabolism in the spinal canal or in the central nervous system.

It seemed possible that an examination of other body fluids low in cells might help in interpreting the results obtained in the study of the spinal fluid. In the present note the results of studies of 6 sterile, approximately normal specimens of pericardial fluid are presented. The material analyzed was removed at postmortem examinations. The number of cells present was never great, it ranged between 600 and 1,500 per cubic millimeter. The cells were almost wholly endothelial in nature. Erythrocytes were absent or present only in small numbers. The most marked chemical difference between these pericardial fluids and the spinal fluids studied previously was due to the presence in the pericardial fluids of relatively large amounts of protein. The concentration of this substance ranged from 1 to 2.6 per cent and averaged 1.6 per cent. The total sugar (dextrose) was determined by the method of Folin and Wu after removing the protein and interfering reducing substances by the zinc precipitation technic of Somogyi. The concentration of sugar varied markedly. It ranged from 75 to 106 mg per hundred cubic centimeters and averaged 113 mg per hundred cubic centimeters. There was no parallelism between the amount of protein and the amount of sugar in the different specimens. Fructose was determined by Roe's application of the resorcinol reaction. The results were corrected for the slight color given by the dextrose present. The average amount was 0.4 mg per hundred cubic centimeters, and the range was from 0 to 1.5 mg per hundred cubic centimeters. Three specimens contained no measurable amount of the sugar. These concentrations are much lower than those previously found in spinal fluid, which averaged 2.9 mg per hundred cubic centimeters. They are approximately the same as the results of blood analyses, which ranged from 0 to 1.9 mg per hundred cubic centimeters and gave an average value of 0.5 mg per hundred cubic centimeter. In these pericardial fluids there was no parallelism between the dextrose and the fructose similar to that observed in spinal fluid.

Confirmation of the nature of the sugars was furnished by a short (twenty minute) incubation with packed yeast cells. The fructose (compound giving the resorcinol reaction) was entirely destroyed by this procedure. The dextrose (total reducing compounds) was almost wholly removed by it. The nondextrose-reducing substances in pericardial fluid, as determined by the Folin-Wu copper reagent after purification with zinc and sodium hydroxide, were equivalent approximately to 3 mg of dextrose per hundred cubic centimeters of fluid. Practically all of the reducing compounds in pericardial fluid are destroyed readily by incubation with yeast cells for short periods of time. Fructose is present in such fluids in approximately the same concentrations as in blood. The results suggest that the presence of fairly large amounts of fructose in the spinal fluid is due to reactions within the fluid itself or to some effect of enzymes or of metabolism of the tissues adjoining the spinal canal.

#### CHOLANGIOMA IN HEPATOLITHIASIS S. SANES and JAMES D. MACCALLUM

From the standpoint of its pathogenesis, primary carcinoma of the liver, including both hepatoma and cholangioma, has been linked to a number of pre-existing lesions, e. g., cirrhosis, parasitic infestation, congenital rests, chronic venous congestion, cholangitis and hepatolithiasis.

We report the rare occurrence of cholangioma in hepatolithiasis associated with cholangitis and cholestasis. The neoplasm was discovered incidentally at autopsy.

A white woman aged 61 years, who had been a diabetic patient for four years, was admitted to the hospital because of a stupor of four days' duration. No history of jaundice was obtained. Death occurred within twelve hours.

The pertinent pathologic observations included early diffuse and patchy pneumonia, hepatolithiasis with dilatation of the intrahepatic bile ducts, which contained brown sandy mucopurulent bile, with several bile ducts showing papillary formations of the lining, small papillary and solid bile duct carcinoma (cholangioma) in the right lobe of the liver, chronic cholangitis of extrahepatic ducts, with choledocholithiasis and dilatation, chronic cholecystitis and cholelithiasis, dilatation of the pancreatic duct, with atrophy of the parenchyma and interstitial fibrosis, atrophy of the brain.

Grossly there was found in the anterosuperior and medial part of the right lobe of the liver a dilated duct which showed at and proximal to the site of lodgment of a stone distinct papillary proliferations into the lumen. On further gross sections through the duct the lumen was practically entirely filled with papillary growth, and the wall was invaded. Surrounding the duct were solid white-yellow nodules. The site of the tumor on transection measured 2.5 by 2.5 cm.

Microscopically, the tumor proved to be an invading papillary and glandular bile duct carcinoma (cholangioma) with solid formations of alveolar and trabecular types. It was associated with hepatolithiasis and with cholangitis and pericholangitis, which showed papillomatous and adenomatous proliferations in the mucosa and in the walls of ducts near the carcinoma and distant from it. Metastases in lymph nodes were not demonstrated.

The question of the pathogenic relationship between hepatolithiasis with cholangitis and cholestasis and cholangioma was discussed.

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## NEW ENGLAND PATHOLOGICAL SOCIETY

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*Annual Meeting, May 18, 1939*

GRANVILLE A. BENNETT, *Secretary*

VIRUS AND BACTERIAL INFECTION OF THE CHICK EMBRYO ERNEST W. GOODPASTURE, Nashville, Tenn. (by invitation)

It is often difficult to find or to procure suitable susceptible experimental animals for use in studies of virus diseases. Viruses as a class are very selective in their requirements for growth. So far as is known, an intracellular environment is necessary for their multiplication, and in some instances only one type of cell of a susceptible host can serve to promote their growth. The need for a host susceptible to many viruses and easy of access has been met to a considerable extent by the embryo of the developing egg.

The cells of the chick embryo have been found to be susceptible to infection by a much greater number of viruses than those of any other animal. At least 15 viruses of man and 12 indigenous to other animals have been successfully used to infect chick embryos.

The chick embryo method has been employed for the preparation of various prophylactic vaccines, namely, those against smallpox, yellow fever, equine encephalomyelitis, fowlpox and laryngotracheitis of fowls.

It has been demonstrated also that certain viruses cultivated by serial passage in chick embryos, while gaining virulence for the embryo, lose virulence for their native host. This occurs with the viruses of human influenza, fowl bronchitis and

herpes simplex. The attenuation thus induced is favorable to the discovery of other vaccines of prophylactic usefulness.

By grafting skin from man or other animal onto the chorioallantois of the chick embryo it is possible to study virus infection and to analyze some of the processes of immunity.

Bacterial infection may likewise be studied in chick embryos because these developing animals are very susceptible to many living pathogenic agents. In fact the chick embryo is the only host in which epidemic meningitis has been induced and its pathogenesis studied experimentally (observations by G. John Buddingh and Alice Polk, unpublished). Lesions simulating those found in human beings with whooping cough may be induced in the respiratory tract of the chick embryo by inoculating it with the bacillus of whooping cough. It is possible by this means also to study such human infections as diphtheria, typhoid fever, undulant fever and others.

The infected chick embryo, furthermore, offers excellent experimental ground for the study of the effects on infection of specific serums and other chemical agents and for assaying the effectiveness of such therapeutic agents.

These matters are discussed in some detail and are exemplified by suitable experimental illustrations.

#### DISCUSSION

ROX FOTHERGILL. It has been a great pleasure for me to hear from Dr. Goodpasture concerning a technic developed by himself, which has been such a useful tool to so many of us. He has surveyed the field of usefulness so completely that one can add but little. I should like to point out one or two things that may be of some interest. In the first place this technic may be particularly useful in the diagnosis of certain virus diseases because in certain instances the egg is more susceptible to infection by small doses of virus than is the usual experimental animal, therefore, one may be able to detect smaller amounts of virus. One feels inclined to emphasize the practical importance of this technic with respect to the production of certain types of vaccine, particularly the production of vaccine for equine encephalomyelitis. Only within the past week a report was received from the United States Bureau of Animal Industry in which it was stated that during the past year, the first year of application of this method to the production of equine vaccine, over 1,600,000 10 cc doses were produced. Another point of some theoretic interest is that this method in respect to certain viruses has permitted one to obtain virus in greater concentrations than previously could be obtained from infected animal tissue. This has permitted a rather definite answer to one controversial problem, i. e., whether or not a virus inactivated by solution of formaldehyde U. S. P. is still immunogenic. Certainly it has been amply demonstrated that the vaccine for equine encephalomyelitis produced in this manner and formaldehydized is abundantly immunogenic. Another possibility of such a method which might be mentioned is that because of the high concentration of virus obtainable from diseased chick tissue there is a possibility of preparing some of the animal pathogenic viruses in a relatively pure state. Stanley has purified a plant virus, but until recently this has not been accomplished with an animal virus. Beard, Wyckoff and others have treated chick tissue equine virus in the ultracentrifuge and obtained a homogeneous sediment which could not be obtained from any other diseased tissue.

E. F. RUSSELL. At the present time how is the smallpox vaccine used?

CONRAD WESSELHOEFT. It was Dr. Goodpasture and his associates who first definitely isolated the virus of mumps. It is to be hoped that his work with the chick embryo will throw some light on the relation between the neurotropic and the cytotropic action of the mumps virus, thus giving us a better understanding of this disease. Another controversial subject which would lend itself to this investigation is whether chickenpox and herpes zoster are related.



JOHN ENDERS It is extremely interesting that the heterogenous tissue should grow on the membrane, and that of course suggests that possibly species antigenic differences have not become fully developed in the embryo I should like to ask whether there has been any investigation of this problem, i e, whether comparative tests of the immunologic specificity of chick embryonic tissue and of the fowl tissues after hatching have been carried out

F P MCCARTHY Have you had any experience as to the results of the use of smallpox vaccine virus in the treatment of recurrent herpes simplex? Some dermatologists are using from six to eight doses of subcutaneous or intradermal vaccine virus in the treatment of herpes simplex, with apparently good results It is a lesion which has bothered dermatologists over a period of years Recently good results have been obtained from that type of therapy

A W SELLARDS I am glad to add my appreciation of Dr Goodpasture's contributions Last summer in Europe there was veritable enthusiasm at the Pasteur Institute in Paris because at last the problem of a bacteria-free vaccine virus had been solved through cultivation of the virus on the chick embryo In Antwerp the Prince Leopold Institute was pleased because at last the chick embryo provided vaccine virus suitable for use under tropical conditions in the Belgian Congo The method enjoys much favor in London, at Hampstead, in the study of virus diseases

I am sure that all share the astonishment in seeing the specific lesions of meningococcic and pertussis infections reproduced for the first time in the chick embryo and that all share the pleasure of seeing the study of infectious diseases placed on a sound basis through the efforts of the pathologists

E A SULLIVAN Did you ever use this method for the study of anaerobes?

ROBERT FIENBERG Were any neoplastic changes observed?

S B WOLBACH How far up in the scales of size do you include organisms under the term "virus"? When I studied bacteriology all organisms were viruses, we spoke of the virus of tuberculosis, then we said "filtrable virus" Now the plain term "virus" in the minds of most persons indicates a filtrable micro-organism

ERNEST W GOODPASTURE Regarding Dr Wolbach's question There is every reason to believe that minute bodies are associated with certain viruses It cannot be definitely stated whether these bodies represent the virus or whether they have a virus so closely associated with them that it is not possible to separate them One is amply justified in considering the elementary bodies which are components of the inclusions of cowpox and smallpox as the active agents of these diseases In regard to what virus is, however, I do not know, and I do not believe anybody else does It is useless to argue whether the viruses are living parasites or not As far as any one knows, they need the life processes of cells for their reproduction When one talks of the phenomena of life, the term in regard to particles as small as virus particles must, I think, have no significance There is one important thing to remember, and that is that the viruses, as far as one knows, and the evidence is very strong, are not filtrable forms of any known bacteria

Dr Russell asked about the use of chick embryo vaccine It can be used either by injection or by inunction after scarification My associates and I have used the latter procedure entirely in conformity with the established practice for calf vaccine The solution of the problem of human vaccination with chick vaccine is not in sight I believe that in the course of time this method will supplant methods that have been satisfactory over a number of years There have been some rather severe local reactions reported with chick vaccine, also greater scar formation, with occasional keloids Dr Harrison questions the use of chick vaccine My point of view is that, as with calf vaccine, one must consider the strain used Dr Buddingh carried the Levaditi strain of neurovaccine and a dermal strain through fifty generations in embryos The Levaditi strain, which is a testicular neurotropic virus, has a tendency to infect the endothelium and connective tissue That is why one gets large hemorrhagic lesions in rabbits These qualities are maintained in the chick embryo, and although at the end of fifty passages this

strain has lost some of its virulence, it still produces hemorrhagic lesions. The dermal strain affected the ectoderm but did not produce hemorrhagic lesions. Manufacturers of vaccine should realize that the strain of virus used is important, and there is no reason to believe that once a good strain of vaccine is obtained it should acquire undesirable qualities by chick embryo passage. A chick embryo vaccine established in the laboratory of the department of pathology of the school of medicine of Vanderbilt University and maintained for seven years shows none of the qualities of neurovaccines. If it is put into the brain of a rabbit, the rabbit does not acquire meningitis. Inoculated in the skin, it produces lesions without hemorrhage. If one has a good strain to start with, there is no reason to believe that good qualities will be lost and bad qualities acquired. A vaccine which produces undesirable lesions may appear on the market. I think this is due to the strain of the virus used and not to the method of production.

Since it is possible to produce with one chick embryo more than a thousand standard doses, based on titration on the rabbit's skin, I think chick vaccine will be used, but I do not think it should have any bad qualities.

Regarding species specificity. I did not have time to go into the history of the development of the chick embryo method, but it is well to remember that it was used in the study of the Rous tumor. Tumor cells have been inoculated into the embryo, with "takes" in the membrane more pronounced than those in the embryo. Following these studies, Dr. James Murphy used the chick embryo membrane method in transplanting tumors. He transplanted cancers of mice and rats, which took perfectly well and grew up to the eighteenth or nineteenth day. The newly hatched chick is entirely resistant to such tumors. The embryo does not seem to have acquired so-called species specificity. It does not respond by the production of immune substances as does the adult.

It has been shown that if a hen is immunized to a certain toxin the antitoxin which develops in the hen will concentrate in the yolk of the egg and that the embryo of the egg from the immunized hen is then not susceptible to the toxin.

In answer to the last question. There are some reports in the literature of good effects from vaccinia in recurrent herpes, but I have had no experience along that line. There have been no experiments that I am aware of on the culture of anaerobic organisms in chick embryos. Successful results have occurred with trypanosomes, certain spirochetes, several of the fungi and some of the protozoan parasites.

## Book Reviews

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**Report of the Medical Research Council for the Year 1937-1938** Presented by the Lord President of the Council to Parliament by Command of His Majesty, February 1939 Paper Pp 221 Price, \$1.05 New York British Library of Information London His Majesty's Stationery Office, 1939

First comes a statement of the Committee of the Privy Council for Medical Research which explains in a general way how the grant of £195,000 (about \$975,000) by Parliament for the year ending Sept 30, 1938, was allocated for research. Then comes the report of the Medical Research Council. In the introduction to the report are discussed such topics as the practical application of new knowledge, research policies, investigations for government departments, the treatment of cancer by radium and research on hormones. Subsequent parts deal with the National Institute for Medical Research and details of the work carried on there by some forty investigators, the determination of biologic standards and methods of biologic assay and measurement, the work of the clinical research units in three London hospitals and at the Royal Infirmary in Edinburgh, the external scientific staff and its investigations, the research aided by grants to workers in various places and in various branches of medicine, research in the field of tropical medicine, industrial health, traveling fellowships, personnel and representation. An appendix gives the names of committees for the special subjects and also indexes of scientific subjects, institutions and personal names. Finally comes a list of publications. The long research front outlined in the report represents clinical and experimental medicine, with special attention to virus diseases, endocrinology, chemotherapy and cancer. Research on a huge scale is conducted under centralized direction but without dictation to the individual worker. The report merits the attention of all who are concerned in an active way with the advance of scientific medicine.

**Failure of the Circulation** Tinsley Randolph Harrison, M.D., Associate Professor of Medicine, Vanderbilt University School of Medicine, Nashville, Tenn. Second edition, revised Cloth Pp 495, with 60 illustrations Price \$4.50 Baltimore Williams & Wilkins Company, 1939

The appearance of a second edition of Harrison's book is evidence of the success of the first edition of only four years ago. The new volume is more than a mere reprint, there has been much revision, as is shown by rearrangement of topics and by numerous additions to the text. The most significant change is the expansion of the discussion of angina pectoris. This includes not only the more recent advances in the physiology and pharmacology of the circulation but the newer views as to the clinical features and treatment of this most important type of acute forward failure of the heart. The whole topic is brought quite up to date. It may be noted incidentally that one is pleased to see the simpler terms "forward failure" and "backward failure" replace the less familiar and rather pretentious "hypokinetic syndrome" and "dyskinetic syndrome." As Harrison says, knowledge of the phenomena of heart disease is more advanced than knowledge of its causes. This means that physicians are often compelled to treat disturbances of function rather than to prevent or overcome underlying causes. One must, therefore, approve the persistent zeal of the Vanderbilt group who are attacking these problems from the standpoint of factual clinical and necropsy manifestations but who at the same time are striving, by physiologic, chemical and bacteriologic experimental methods in the ward and the animal laboratory, to understand better the fundamental processes that are involved in normal circulation and its failure.

**Lehrbuch der allgemeinen Pathologie und der pathologischen Anatomie.**

H Ribbert Twelfth edition Edited by Prof Dr H Hamperl, Prosektor am pathologischen Institut der Universität, Berlin Paper Pp 634, with 700 illustrations Price, 27 reichmarks Berlin F C W Vogel, 1939

Hamperl's new twelfth edition of Ribbert's book appears to be a good general text for students of medicine. Some of the sections on general pathologic processes are excellent, notably that on thrombosis and those on some of the inflammations. The section on tumors is brief but appears reasonably comprehensive. The illustrations include many excellent photographs as well as many diagrams and drawings. As might be expected from the extent of the text (634 pages, including 700 illustrations), many subjects are referred to very briefly or omitted altogether. One finds no reference to tularemia, typhus or plague and only slight reference to many of the other infectious diseases. The hepatic changes in Weil's disease are mentioned but not the characteristic interstitial nephritis. The scope of the book is too restricted to permit much use of the matter as a source of reference by pathologists.

## Books Received

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MEDICAL JURISPRUDENCE AND TOXICOLOGY William D McNally, B A , M D , Assistant Professor of Medicine and Lecturer in Toxicology, Rush Medical College, University of Chicago, Chicago Cloth Pp 386, with 23 illustrations Price \$3 75 Philadelphia and London W B Saunders Company, 1939

MEDICAL MICROBIOLOGY Kenneth L Vurdon, Ph B , M S , Ph D , Assistant Professor of Immunology and Bacteriology, Louisiana State University School of Medicine, New Orleans Cloth Pp 73, with 120 illustrations Price \$4 50 New York The Macmillan Company, 1939

CANCER HANDBOOK OF THE TUMOR CLINIC, STANFORD UNIVERSITY SCHOOL OF MEDICINE Edited by Eric Liljencrantz, M D , Chief of Tumor Clinic, Stanford University School of Medicine, San Francisco Cloth Pp 114, with 50 illustrations Price \$3 Stanford University, Calif Stanford University Press, 1939

SYMPOSIUM ON THE SYNAPSE Herbert S Gasser, Joseph Erlanger, Detlev W Bronk, Rafael Lorente de No and Alexander Forbes (Reprinted from the *Journal of Neurophysiology* [2 361-472, 1939] ) Cloth Pp 361-474 Price \$2 Springfield, Ill , and Baltimore, Md Charles C Thomas, Publisher, 1939

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